Biopolymers: Biomedical and Environmental Applications

Scrivener Publishing
3 Winter Street, Suite 3
Salem, MA 01970

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Biopolymers: Biomedical and Environmental Applications

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Scrivener

WILEY

Co-published by John Wiley & Sons, Inc. Hoboken, New Jersey, and Scrivener Publishing LLC, Salem, Massachusetts.
Published simultaneously in Canada.

Library of Congress Cataloging-in-Publication Data:

ISBN 978-0-470-63923-8

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

Contents

Part 2: Bioplastics and Biocomposites

Preface

There is currently a tremendous interest in the latest information concerning polymer related topics. Experts predict the future availability of fossil resources (oil, natural gas and coal), which are not renewable, varies between one and three generations. Keeping in mind the deteriorating environmental conditions caused by many factors including advancements in science and technology, population expansion, global warming, etc, researchers all over the world have recently focused on biopolymers from renewable resources with much success.

This book focuses on different biopolymers and their applications in various fields. It highlights recent advances in technology in many areas from chemical synthesis and biosynthesis to end-user applications. These areas have not been covered in a single book before, and include information on biopolymers from chemical and biotechnological modifications, material structures, processing, characterization, properties, and applications.

Chapters cover nearly every conceivable topic related to polysaccharides, such as biofibers, bioplastics, biocomposites, natural rubbers, proteins, gums, and bacterial polymers. Given the global context it does not seem preposterous to consider the materials discussed as the polymers of the future.

The book distills recent research conducted by the scientific community. It is arranged in four parts. Part I, Polysaccharides, covers hyaluronic acid, chitin and chitosan, starch and other natural polysaccharides. Polysaccharides have received more attention due to their numerous advantages such as their renewability, non-toxicity, biodegradability and ready availability. This interest has resulted in a great revolution leading to polysaccharides becoming on par with, and even superior to, synthetic materials. That is why a plethora of research studies have been undertaken to understand the potential of these natural polymers.

Hyaluronic acid is a linear polysaccharide formed from disaccharide units containing N-acetyl-D-glucosamine and glucuronic acid. Since it is present in almost all biological fluids and tissues, hyaluronic acid-based materials are very useful in biomedical applications. After cellulose, chitin is the second most abundant natural polysaccharide resource on earth. Chitin and its de-acetylated derivative chitosan are natural polymers composed of N-acetylglucosamine and glucosamine. Both chitin and chitosan have excellent properties such as biodegradability, biocompatibility, non-toxicity, hemostatic activity and antimicrobial activity. Chitin and its derivatives are widely used in various fields of medicine.

Polysaccharides and their graft copolymers are finding extensive applications in diversified fields. The graft copolymerized and crosslinked polysaccharides are cost effective, biodegradable and quite efficient for use in technological processes. The end products obtained have improved properties that can be used in fields such as sustained drug delivery systems, controlled release of insecticides and pesticides to protect plants in agricultural and horticultural practices, release of water for plants during drought conditions, water treatment and membrane technology. Modified polysaccharides have found applications from permselective membranes to ionically conductive membranes for fuel cells.

Starch is the major carbohydrate reserve in higher plants and has been one of the materials of choice since the early days of human technology. Recently, starch gained new importance as a raw material in the production of bioplastics, in particular for use in the synthesis of monomers to produce polymers such as poly(lactic acid), and after chemical modification and thermomechanical processing, to produce the so-called thermoplastic starch.

Part II discusses bioplastics and biocomposites. One of the main environmental problems in industrial development is plastic waste and its disposal. An enormous part of scientific research has been directed towards environmentally benevolent bioplastics that can easily be degraded or bio-assimilated. High performance bio-based composites (biocomposites) are very economical and open up a wide range of applications.

Part III covers different biopolymers such as gums, proteins, natural rubbers and bacterial polymers and some of their applications. The genus *Cassia* has been the centre of attraction for many phytochemists throughout the world, especially in Asia. *Cassia* plants are a known source of seed gums which are usually galactomannans having close structural resemblance to many of the commercial seed gums, such as guar and locust bean gums, and are considered as non-conventional renewable reservoirs for the galactomannan seed gums. Thus, properties of *Cassia* seed gums in general can be tailored by chemical modification whereupon they can be exploited as useful dye flocculants and heavy metal adsorbents depending upon their solubility in water. Though galactomannans from *Cassia* seeds are nonionic polysaccharides, their adsorption performance is comparable with that of chitin and chitosan, and superior to other polysaccharides.

Gum Arabica is a natural plant gum that exudates a carbohydrate type and is an electroactive biopolymer. *Gum Arabica* and its complexes have potential applications in developing ionic devices such as batteries, sensors, bio-sensors, and other electronic applications, in addition to solar material, energy storage material and nanoscience. Biopolymers obtained from bacteria are rapidly emerging because they are biodegradable and available in abundance. Simple methods are being developed to grow and harvest the polymers to exploit them for numerous industrial and biomedical applications. Electronic structures and conduction properties of biopolymers are also discussed in Part III.

Part IV includes applications of various biopolymers such as seed coating to protect against biotic stress, biosorbent for the organic pollutant, pharmaceutical technology, drug delivery, and gene therapy.

Discussions in this book regarding the very important issues and topics related to biopolymers should be useful to those in the scientific community such as,

scientists, academicians, research scholars, polymer engineers and specialists in other industries. The book also acts as a support for undergraduate and postgraduate students in the institutes of polymer and technology and other technical institutes. We hope it will be an exceptional book with important contributions from well-known experts from all over the world.

Both Editors would like to express their gratitude for all the excellent contributions made by the contributors to this book. We would also like to thank all who helped in the editorial work as well.

Susheel Kalia
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April 2011

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PART 1
POLYSACCHARIDES

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (3–34)

1

Hyaluronic Acid: A Natural Biopolymer†

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Abstract

This chapter gives a brief overview on glycosaminoglycans, with a special focus on hyaluronic acid/hyaluronan – its structure, occurrence, and function, along with its broadly enlarging applications. Hyaluronan biosynthesis, catabolism, and degradation as well as its technological input in regenerative medicine where hyaluronan is applied at viscosurgery, viscoprotection, and viscosupplementation is presented as well. Also, special interest is focused on elucidating cellular mechanisms such as the effects of chemical pathways-driven oxidative stress.

Keywords: Glycosaminoglycans, hyaluronan biosynthesis, hyaluronan catabolism, hyaluronan oxidative degradation, inflammation, inflammatory mediators, regenerative medicine

Abbreviations: BMP: bone morphogenic protein, CD: cluster of differentiation, CS: chondroitin sulfate, DFO: deferoxamine, DMF: dimethylformamide, DS: dermatan sulfate, ECM: extracellular matrix, ESR: electron spin resonance, GAG(s): glycosaminoglycan(s), GalNAc: *N*-acetyl-D-galactosamine, GlcA: D-glucuronic acid, Gy: gray (unit of the energy dose: J/kg), HA: hyaluronic acid (hyaluronan, hyaluronate), HAS(s): hyaluronan synthase(s), Hep: heparin, HPLC: high performance liquid chromatography, HS: heparan sulfate, HYAL(s): hyaluronidase(s), IdoA: L-iduronic acid, IFN-γ: interferon gamma, IL-1β: interleukin beta, k: second order rate constant, KS: keratan sulfate, MPO: myeloperoxidase, mRNA: messenger ribonucleic acid, MS: mass spectrometry, NADPH: nicotinamide-dinucleotide phosphate, NMR: nuclear magnetic resonance, OA: osteoarthritis, PG(s): proteoglycan(s), pK_a: natural logarithm of acid/base equilibrium, PMA: phorbol-myristoyl acetate, ppm: parts per million,

†Dedicated to Dr. Grigorij Kogan in appreciation of his significant contribution to the studies of polysaccharides.

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (3–34)

RA: rheumatoid arthritis, RNS: reactive nitrogen species, ROS: reactive oxygen species, SF: synovial fluid, SOD: superoxide dismutase, *SPAM1*: sperm adhesion molecule-1, TGF-β: transforming growth factor beta, TNB: 5-thio-2-nitrobenzoic acid, TNF-α: tumor necrosis factor alpha, UDP: uridine diphosphate

1.1 Glycosaminoglycans

Glycosaminoglycans (GAGs) are natural, very complex, unbranched, polydisperse polysaccharides composed of disaccharide units of D-glucuronic acid (GlcA) or L-iduronic acid (IdoA) [keratan sulfate (KS) has galactose instead of uronic acid] linked to a D-glucosamine (GlcN) or D-galactosamine (GalN) residue (Figure 1.1). In general, GAGs are sulfated macromolecules [with the exception of hyaluronic acid (HA)] having different numbers of sulfate groups linked at different positions. They have very heterogeneous structures by considering relative molar mass, charge density and chemical properties generating various biological and pharmacological activities [1, 2]. Based on carbohydrate backbone structure, it is possible to distinguish four classes of GAGs: 1. HA, 2. KS, 3. chondroitin sulfate (CS)/dermatan sulfate (DS), and 4. heparan sulfate (HS)/heparin (Hep). HA is the only GAG containing an unmodified *N*-acetyl-D-glucosamine (GlcNAc)-GlcA repeating unit, while the other polysaccharides are generally modified through post-biosynthetic modifications, such as the addition of *O*-sulfo groups, C5-epimerization to form IdoA residues, and de-*N*-acetylation to produce GlcN-sulfo residues. These macro and micro modifications often play a key role in a wide variety of biological and pharmacological processes [1, 2].

GAG chains are covalently attached (with the exception of HA) at their reducing end to a core protein to produce macromolecules named proteoglycans (PGs) [3–5] localized at cellular and extracellular levels playing structural and regulatory roles due to their interaction with several proteins. In fact, PGs are not only structural components, but they participate in many cellular events and physiological processes, such as cell proliferation and differentiation, cell-cell and cell-matrix interactions [6–8] and are implicated in regulatory functions of development, angiogenesis, axonal growth, cancer progression, microbial pathogenesis, and anticoagulation [1, 2, 6–8] due to the specific interactions between structural GAGs and numerous proteins. As a consequence, these heteropolysaccharides are macromolecules of great importance in the fields of biochemistry, pathology and pharmacology.

KS was extracted and characterized for the first time from the bovine corneal stroma [9] and called KS-I having an alkali-stable bond between GlcNAc and asparagine. Skeletal KS with the alkali-labile bond between GalNAc and serine or threonine was designated as KS-II. This skeletal type has been further subclassified into articular, KS-IIA and KS-IIB [10], for the presence on the former of α(1-3)-fucose and α(2-6)-N-acetylneuraminic acid absent in the latter. The repeating disaccharide unit of KS [Gal (β1$\rightarrow$4) GlcNAc (β1$\rightarrow$3)]$_n$ contains a galactose residue instead of uronic acid and the glycosidic bonds are reversed

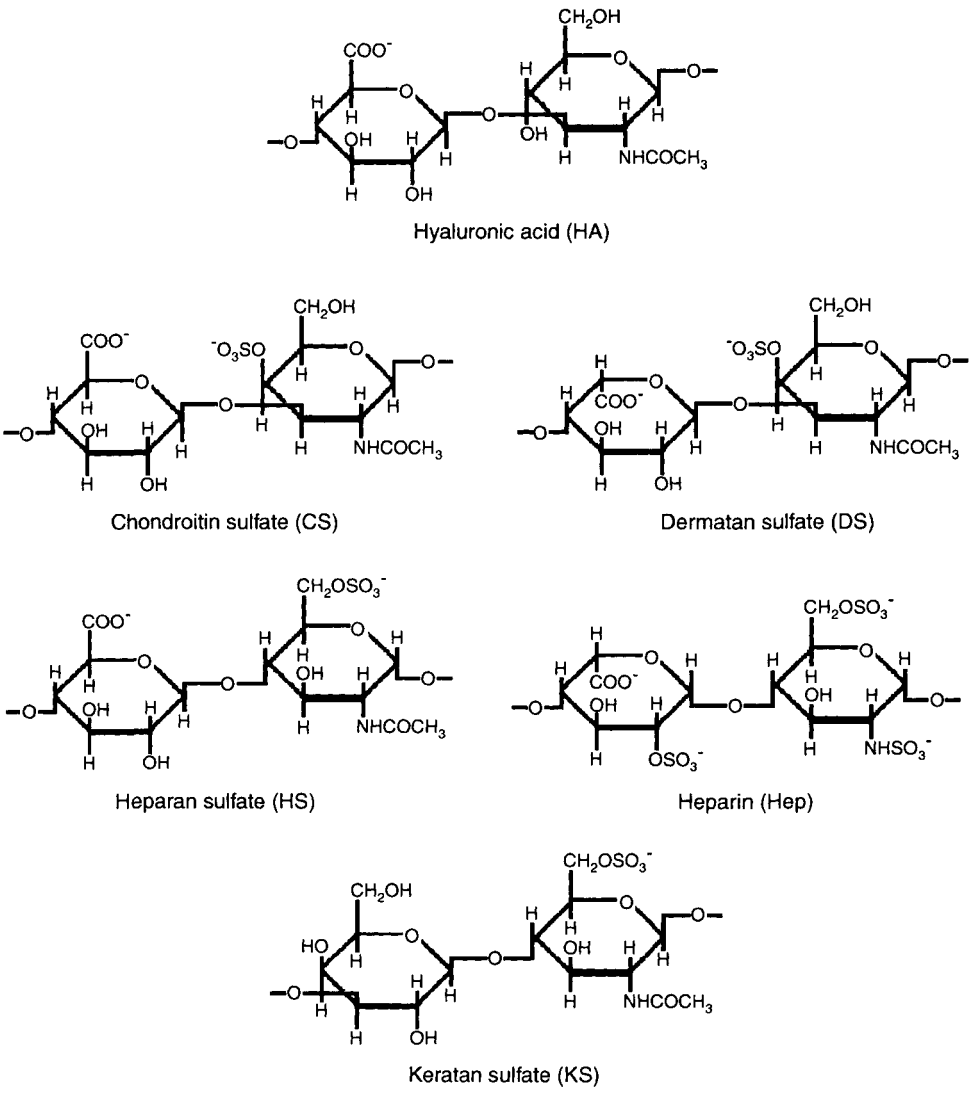


Figure 1.1 Structures of repeat units forming GAGs. Major modifications for each structure are illustrated but minor variations are also possible.

in comparison with HA and CS/DS (Figure 1.1). Sulfate esters are present at the C-6 of one or both of the monosaccharides forming the disaccharide unit, but any other hydroxyl group may carry an esterified sulfate group.

CS and CS B, also known as DS, are constituted by the disaccharide unit [GlcA (β1→3) GalNAc (β1→4)]$_n$ variously sulfated in different positions of the hexosamine unit and/or uronic acid. Some, although relatively few, of these positions remain unsulfated. The regular disaccharide sequence of CS A, chondroitin-4-sulfate, is formed by the repeating unit sulfated in position 4 of the GalNAc unit, while CS C, chondroitin-6-sulfate, is composed of a disaccharide unit sulfated in position 6. Disaccharides with different numbers and

positions of sulfate groups can be located, in different percentages, inside the polysaccharide chains, such as the disulfated disaccharides in which two sulfate groups are *O*-linked in position 2 of GlcA and 6 of GalNAc (disaccharide D) or in position 4 and 6 of GalNAc (disaccharide E) [11].

In the case of DS, further enzymatic modifications complete the final structure, such as C-5 epimerization of GlcA to IdoA, and *O*-sulfation at C-2 of IdoA. As a consequence, polysaccharide chains of DS are formed of a prevailing disaccharide unit [IdoA (β1→3) GalNAc (β1→4)]$_n$ with a minor concentration of disulfated disaccharides, in particular sulfated in position 4 of GalNAc and 2 of the IdoA unit [11] (Figure 1.1). These heterogeneous structures are responsible for the different and more specialized functions of these GAGs. Furthermore, IdoA imparts conformational flexibility to the DS chain altering the shape and spatial orientation of sulfate residues, endowing the chain with a higher negative charge density than the GlcA [11]. Although the principles of the biosynthetic process have not yet been fully elucidated, it is well known that this process results in the generation of highly modified oligosaccharide domains separated by regions of relatively low-degree structural modifications within the polymer chain. Thus, the DS chain has a hybrid co-polymeric structure consisting of low modified (CS) and highly modified (DS) domains [12]. The IdoA-containing units are often sulfated at C-4 of the GalNAc residue, while sulfation at C-6 is frequently associated with GlcA-containing disaccharides [13]. Twenty-three different CS/DS disaccharides have been identified so far [13].

Hep and HS possess a distinctly different repeating disaccharide structure compared with the previous GAGs [GlcA (β1→4) GlcNAc (α1→4)]$_n$ (Fig. 1.1). Hep is sometimes considered to be synonymous with HS, but this is an oversimplification. They have been shown to differ in their degree of sulfation, with Hep being more negatively charged and displaying higher *N*- and *O*-sulfation than HS. They follow different biosynthetic paths in different cells and in different core proteins. The glycosidic linkage between uronic acid and GlcN is (β1→4) instead of (β1→3), and that between GlcN and uronic acid is (α1→4) instead of (β1→4) [6,7,14]. The growing GAG polymer chain is *N*-deacetylated and *N*-sulfated at the glucosamine residues, yielding regions in the chain particularly available for further structural changes, in particular C-5 epimerization of GlcA and *O*-sulfation mainly at C-2 of IdoA and C-6 of glucosamine [14]. Other more infrequent *O*-sulfations occur at C-2 of GlcA and C-3 of *N*-sulfated glucosamine. A few of the glucosamine amino groups may also remain unsubstituted. This process yields hybrid structures with hyper-variable, highly sulfated domains and poorly modified ones. As reported above, Hep has the highest charge density of any known biological macromolecule, while HS is generally less sulfated and possesses lower IdoA content. Both GAGs are highly polydisperse macromolecules, depending on tissue origin and status. Due to their properties, Hep and HS exhibit diverse biological functions and participate in a large number of interactions with other effective extracellular and cell membrane molecules, such as growth factors, virus proteins, enzymes, adhesion proteins, integrins, and thrombin/antithrombin [6, 7, 14].

1.2 Hyaluronic Acid/Hyaluronan – Structure, Occurrence

As stated above, hyaluronic acid (Figure 1.1), also called hyaluronan and sometimes presented as a hyaluronate (poly)anion from the chemical/structural viewpoint, is a non-sulfated GAG, while all other glycosaminoglycans are sulfated polysaccharides (cf. Table 1.1). Another fundamental and remarkable difference between HA and the other GAGs is the mean molar mass of the native biopolymeric chains. While the value of several MDa is the most common one for HA synthesized by hyaluronan synthases (HAS1, HAS2, and HAS3), the molar mass of further glycosaminoglycans, on average, does not exceed the value of 50 kDa [8].

In aqueous solutions, at a physiological pH, HA is represented by negatively charged hyaluronate macromolecules (pK_a = 3.21) [15] with extended conformations. In a polyanionic form, hyaluronan functional groups make the biopolymer so hydrophilic that it binds 1000 times more water than is predicted from its molar mass. The heterogeneity and hydrophilicity of HA facilitate its interaction with a variety of tissue constituents inside and outside the cells. In the extracellular space, HA controls the retention of water, ionic and molecular diffusion and provides a 3D-structural meshwork [16].

HA is omnipresent in almost all biological fluids and tissues of the vertebrates, in which the highest amount is found in the extracellular matrix (ECM) of soft connective tissues [17]. In the skin, for example, there is slightly more than 50% of the total HA content present within the human body. The turnover

Table 1.1 Glycosaminoglycans.

Name	Constituent Sugars	Sulfate Group	Approx. Mean Molar Mass [Da]
Hyaluronan[a]	glucuronic acid glucosamine	–	up to 1×10^7
Chondroitin 4-(6-) sulfates	glucuronic acid galactosamine	+	$10–50 \times 10^3$
Dermatan sulfate	iduronic acid galactosamine	+	$10–50 \times 10^3$
Keratan sulfate	galactose glucosamine	+	$5–15 \times 10^3$
Heparan sulfate	glucuronic and iduronic acid glucosamine	+	$10–50 \times 10^3$
Heparin	glucuronic and iduronic acid glucosamine	+	$5–20 \times 10^3$

[a]At the HA mean molar mass of about 1×10^7 Da, the polymer chain, if it is straightened, would exceed 15 μm

of hyaluronan in most tissues in the body is surprisingly rapid, with the exception of the vitreous body where its half-life is 20–70 days. Typical half-life in skin and pericardium is 2–5 days, whereas it is 0.5–1 day in joints and pleura, and 1–2 h in the anterior chamber of the eye. The synthesis of hyaluronan takes place locally in the tissues, while the breakdown is a process that partly takes place locally in the tissue, but also to a large extent in local lymph nodes and in the endothelial cells of the liver. The catabolism of hyaluronan takes place both by local degradation and drainage *via* the lymphatic system [18].

Generally, two fractions of hyaluronate macromolecules can be characterized in the living vertebrate organisms: (*i*) free, not associated with other tissue constituents such as proteins, and (*ii*) the protein associated fraction of HA [19].

1.3 Hyaluronan Synthases

Hyaluronan is the product of biosynthesis *via* articular chondrocytes and synovial cells on the inner surface of the plasma membrane-bound hyaluronan synthases (HASs) in eukaryotes. This fact has a great importance in physiology and pathology of joint inflammation and cancer progression [20, 21]. While the enzymes hyaluronidase-1 (HYAL1) and hyaluronidase-2 (HYAL2) have both predominant function in the HA catabolism and a high rate of this biopolymer turnover [17], HA itself is synthesized *de novo* in mammals by the three related integral membrane isoenzymes (HAS1, HAS2, HAS3) producing varying chain lengths [20, 22] in a dynamic continuous manner [21]. As atypical for glycosaminoglycans, HA is synthesized at the plasma membrane with the polymer being extended from the reducing end which results in its extrusion from the cell surface [23]. HASs utilize two sugar substrates, uridine diphosphate (UDP)-glucuronic acid and UDP-*N*-acetylglucosamine, to alternately add the corresponding UDP-sugar to the reducing end of the hyaluronan chain with release of the anchoring UDP [24]. Simultaneously with synthesis, the growing hyaluronan chain is translocated through plasma membrane into the extracellular space [25]. Being likely a part of a larger protein complex in a cell organelle regulating enzyme activity, the HASs, however, do not require primers when exerting their activity [17]. To perform specific biological purposes, HASs are responsible for the biosynthesis of various HA size distributions [26]. Depending on the catalytic rate and mode of regulation, HAS1 is the least active, and rules the HA synthesis from 2×10^5 to 2×10^6 Da; HAS2 is more active generating HA greater than 2×10^6 Da – this enzyme is probably responding to oxidative stress-induced increased HA synthesis. The enzyme is also involved in regenerative tissue processes and is also associated with cell migration, invasion, and proliferation. HAS3 is the most active HAS enzyme promoting the synthesis of HA chains from 0.2×10^6 to 2×10^6 Da. The pericellular glycocalyx might be provided by HAS3 products as well as the HA interacting with cell surface receptors [17]. The first HA synthase gene (42 kDa) from Group A *Streptococcus* bacteria was discovered in 1993 [27, 28]. The amount of HA synthesized *in vivo* is postulated that it should be strictly regulated by

a signal receptor CD44 which is a component of some feed-back mechanism [17]. The three HAS enzymes (Figure 1.2) are encoded by the three related tissue- and cell-specific *HAS* genes on three different chromosomes [29, 30]. Comparison of the deduced amino acid sequences exhibits a high degree of homology between the HAS enzymes, particularly HAS2 and HAS3. They all contain seven putative membrane-spanning domains, two of which are located at the *N*-terminal end of the molecules while the other five are located at the C-terminal end [31]. Regarding HA bioproduction, synovial cells preferentially utilize the HAS1 message, whereas chondrocytes and osteosarcoma cells the HAS2 message [20]. Cytokines such as tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and interleukin beta (IL-1β) may modulate HASs transcription activity and the variation of HA levels in normal and oxidative stress-driven fibroblasts. A synergistic effect was observed in normal fibroblasts when concomitantly exposed to the cytokines and oxidative stress; the increase

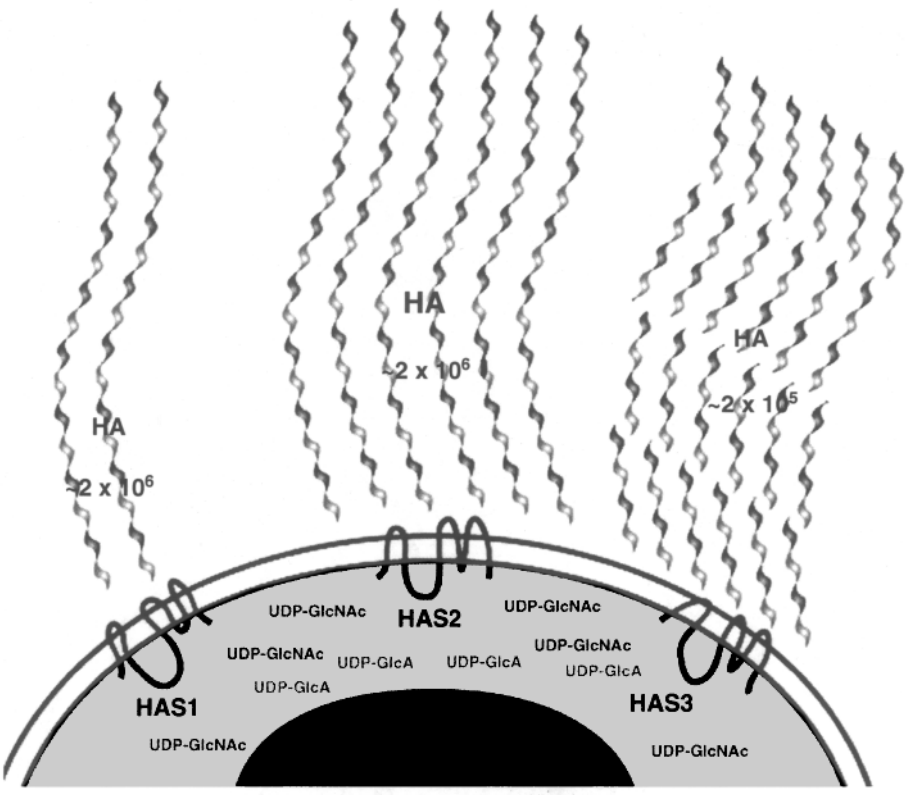


Figure 1.2 Regulation of hyaluronan amount and chain length by expression of a specific HAS protein. Biochemical characterizations of the vertebrate HAS enzymes expressed in mammalian cell culture have revealed similarities and differences between the respective mammalian hyaluronan synthase enzymes. The differences are depicted in this cartoon. HAS1 produces small amounts of high-molar-mass hyaluronan. HAS2 produces significantly more high-molar-mass hyaluronan. HAS3 is the most active of the hyaluronan synthases, yet produces low-molar-mass hyaluronan chains. The physiological significance of these differences in enzymatic activity is not yet known [33].

in HASs mRNA expression was detected. HASs stimulation-derived HA production in reactive oxygen species (ROS)-treated fibroblasts may represent a biological defending system against cell damage caused by ROS [32]. As to the HAS enzymes in synovial fibroblasts in osteoarthritic (OA) and rheumatoid arthritis (RA) patients, HAS1 mRNA is up-regulated by transforming growth factor (TGF-β), whereas HAS3 is up-regulated by TNF-α and IL-1β [17].

1.4 Enzymatic Catabolism of Hyaluronan

Hyaluronan degrading enzymes, the hyaluronidases (HYALs) are, in general, responsible for specific catabolic pathways involved in HA turnover. Karl Meyer was the pioneer scientist who described the *in vivo* occurrence as well as the biochemical functioning of HYALs [34]. The so-called *Meyer's* scheme consists of the three groups of HYALs due to their various endobiotic effects [35]. Bacterial HYALs, functioning as lyases, are β-endo-*N*-glucosaminidases, mammalian HYALs are of the same composition as the first ones, however, they function as hydrolases. The third group of HYALs, composed of hydrolytic β-endoglucuronidase, is related to the vertebrate enzymes. Isolation and characterization of HA degrading enzymes in eukaryotes was accomplished by Frost *et al.* [36]. Being present at subtle concentrations exhibiting high, however, unstable specific activities, HYAL enzymes activity is, during purification, usually kept by protease inhibitors and detergents [17].

Continuously ongoing HA turnover (5g daily from 15g of HA per 70 kg human individual) is a well-established phenomenon and is carried out by a rapid catabolic action of HYALs. HA catabolism is, in fact, realized *via* two simultaneously occurring mechanisms, the enzymatic and chemical (oxidative) scission whose correlative proportion is unknown.

HA catabolism/degradation results in the formation of different HA fragments. The parental biopolymer and the enzyme-mediated HA fragments, regardless of chain length, have both identical chemical structure whereas fragmented chains produced under oxidative stress contain e.g. aldehyde-, hydroperoxide-, and other chemical groups [17].

In humans, the HYALs constitute an enzyme protein family having a high degree of sequence homology. There are six genes tightly clustered at two chromosomal locations with HYAL-like sequences. The three genes, *HYAL1*, *HYAL2* and *HYAL3* coding for HYAL1, HYAL2 and HYAL3, are on chromosome 3p21.3 [37, 38]. They are organized in an extraordinarily complex and overlapping manner in an area densely packed with transcribed genes [39]. Regarding the cluster on chromosome 3p, HYAL1 (55 kDa glycoprotein monomeric unit) and HYAL2 (54 kDa glycoprotein monomeric unit) are probably the major eukaryotic hyaluronidases in connective tissues. HYAL1, being probably a lysosomal enzyme, cleaves HA predominantly to tetrasaccharides [37, 38, 40]. HYAL2 is attached to the plasma membrane *via* a glycosylphosphatidylinositol link. This enzyme cleaves high-molar-mass HA to approximately 20 kDa fragments [41, 42]. Widely expressed HYAL3 enzyme activity and function has not been

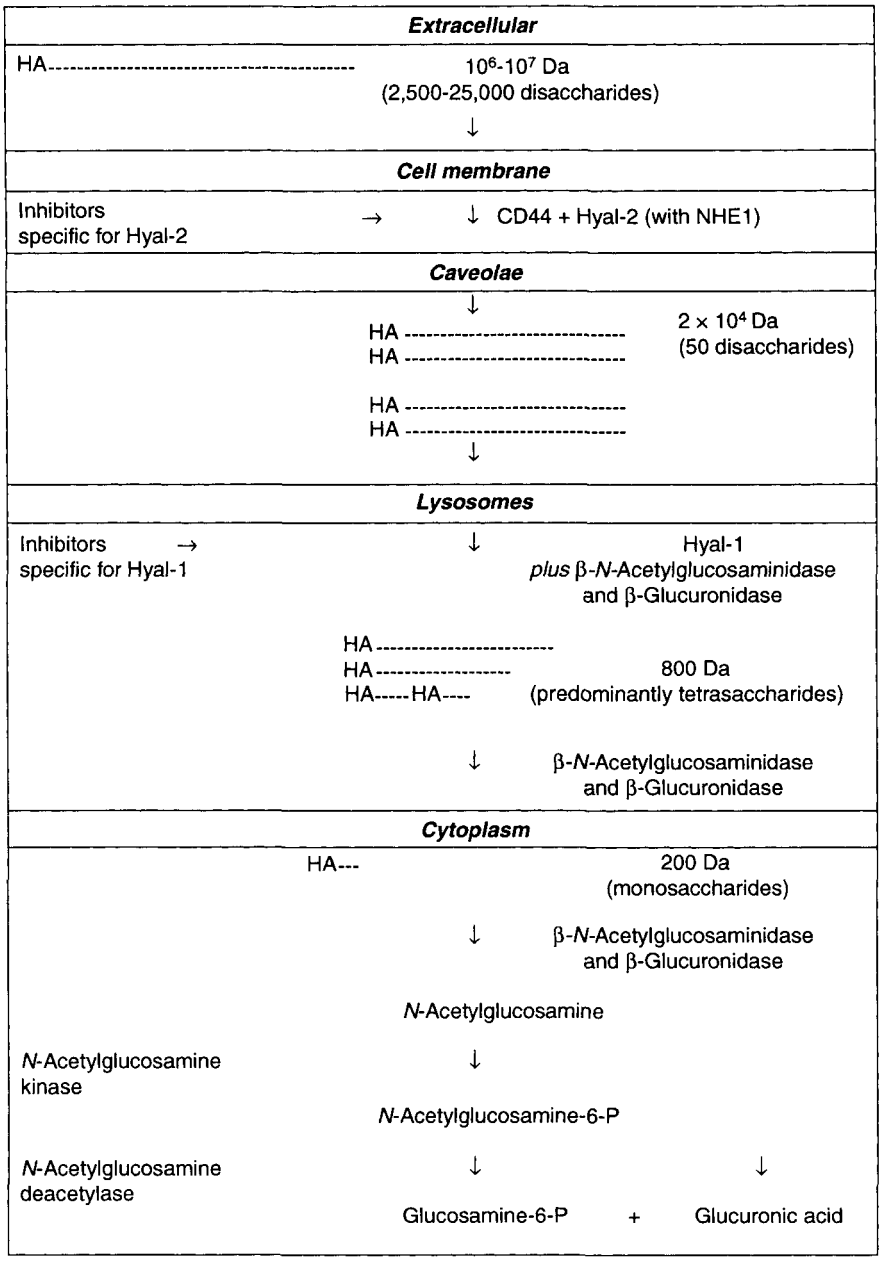
satisfactorily documented [43, 44]. The three genes, *HYAL4*, *PHYAL1* and *SPAM1* (Sperm Adhesion Molecule-1) are clustered in a similar fashion on chromosome 7q31.3, coding respectively for *HYAL4*, a pseudogene transcribed, but not translated in the human, and PH-20. The PH-20 enzyme facilitates penetration of sperm through the cumulus mass surrounding the ovum, and is also necessary for fertilization [45, 46]. It is a multifunctional enzyme protein which has a separate domain bound to zona pellucida. PH-20 can be detected in other positions in the male reproductive tract applying polymerase chain reaction analysis [47], the female genital tract [48], and moreover, in several malignancies [49, 50].

The β-endoglycosidase enzymes, HYAL1 and HYAL2, involved in HA catabolism in connective tissues, are the triggers of HA scission. High-molar-mass HA degradation to individual sugars (Scheme 1.1) is accomplished by the lysosomal exoglycosidases, β-glucuronidase, and β-*N*-acetyl-glucosaminidase. The HYAL enzymes and products of enzyme-like sequences have some other important functions – they also behave as receptors [51], or as adhesion/anti-adhesion molecules.

A pathway of intermediary metabolism is depicted in Figure 1.3 involving the HA catabolism. The cell surface hyaluronan receptor, CD44, two hyaluronidases, HYAL-1 and HYAL-2, and two lysosomal enzymes, β-glucuronidase and β-*N*-acetyl-glucosaminidase, are involved. This metabolic cascade begins in lipid raft invaginations at the cell membrane surface. Degradation of the high-molar-mass extracellular HA occurs in a series of discrete steps generating HA chains of decreasing sizes. The biological functions of the oligomers at each quantum step differ widely, from the space-filling, hydrating, anti-angiogenic, immunosuppressive 104-kDa extracellular biopolymer, to 20-kDa intermediate polymers that are highly angiogenic, immunostimulatory, and inflammatory. This is followed by degradation to small oligomers that can induce heat shock proteins and that are anti-apoptotic. The single sugar products, D-glucuronic acid and a glucosamine derivative are released from lysosomes to the cytoplasm, where they become available for other metabolic cycles [52].

1.5 Oxidative Degradation of Hyaluronan

There are basically two different reasons why the degradation of hyaluronan by free radicals is of interest: (a) many inflammatory diseases (for instance, RA or OA) are accompanied by the generation of free radicals that may lead to tissue damage [54] and (b) this is a frequently used method to generate smaller fragments of HA, i.e. with a defined number of polymer repeating units [55]. We especially focus here on the free radical-induced degradation of HA which is particularly useful if chemically modified HA is to be investigated because many modifications of HA (e.g. the introduction of sulfate groups) lead to partial or even complete inhibition of HA-depleting enzymes such as hyaluronidase(s) [56].



Scheme 1.1 Hyaluronan catabolism[a].
[a]A proposed mechanism for HA catabolism: high-molar-mass extracellular biopolymer is gradually degraded into single sugars available to enter corresponding metabolic pathways.
Abbreviation: NHE1, Na^+/H^+ exchanger isoform 1.

Before some basic reactions are discussed in more detail, one short definition of the terms "free radicals" and "reactive oxygen species" is needed [57]: Basically, all "free radicals" are "ROS", but not every "ROS" is a "free radical". Free radicals are always paramagnetic compounds due to the presence of an

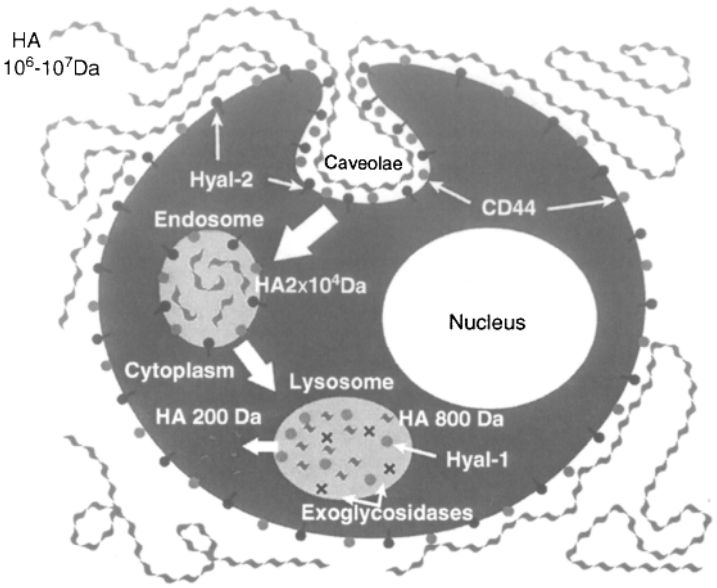


Figure 1.3 Putative metabolic scheme for hyaluronan degradation [53].

unpaired electron ("•"). Therefore, the compounds such as hypochlorous acid (HOCl) or hydrogen peroxide (H_2O_2) are ROS but do not represent free radicals because they lack the unpaired electron.

We focus here primarily on ROS that do also play a major role under *in vivo*, e.g. inflammatory conditions. Although there are many further species that can be generated by means of chemical reactions and are basically capable of degrading HA under *in vitro* conditions [58], these species are not discussed here in more detail. Additionally, superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are not discussed. Although these are very important species, they possess only low reactivity and do not provide major oxidative damages to biomolecules.

The *in vivo* generation of all relevant species is discussed in the next paragraph, while some selected laboratory scale-methods to study ROS generation are also discussed.

1.5.1 Reaction of HA with HO• Radicals

Hydroxyl radicals (HO•) represent one of the most reactive species and may be generated *in vitro* by the Fe^{2+} ion-induced decomposition of H_2O_2 [59], by the light-induced scission of H_2O_2 or by H_2O radiolysis [60]. Water hydrolysis is unequivocally the most specific method of HO• radical generation and the radical yield can be easily altered by the applied energy dose [61].

HO• radicals react in a diffusion-controlled manner ($k \approx 6 \times 10^9$ l mol^{-1} s^{-1}) with virtually all compounds containing C-H groups under the abstraction of one hydrogen (H•) radical [62] leading to the generation of the corresponding alkyl

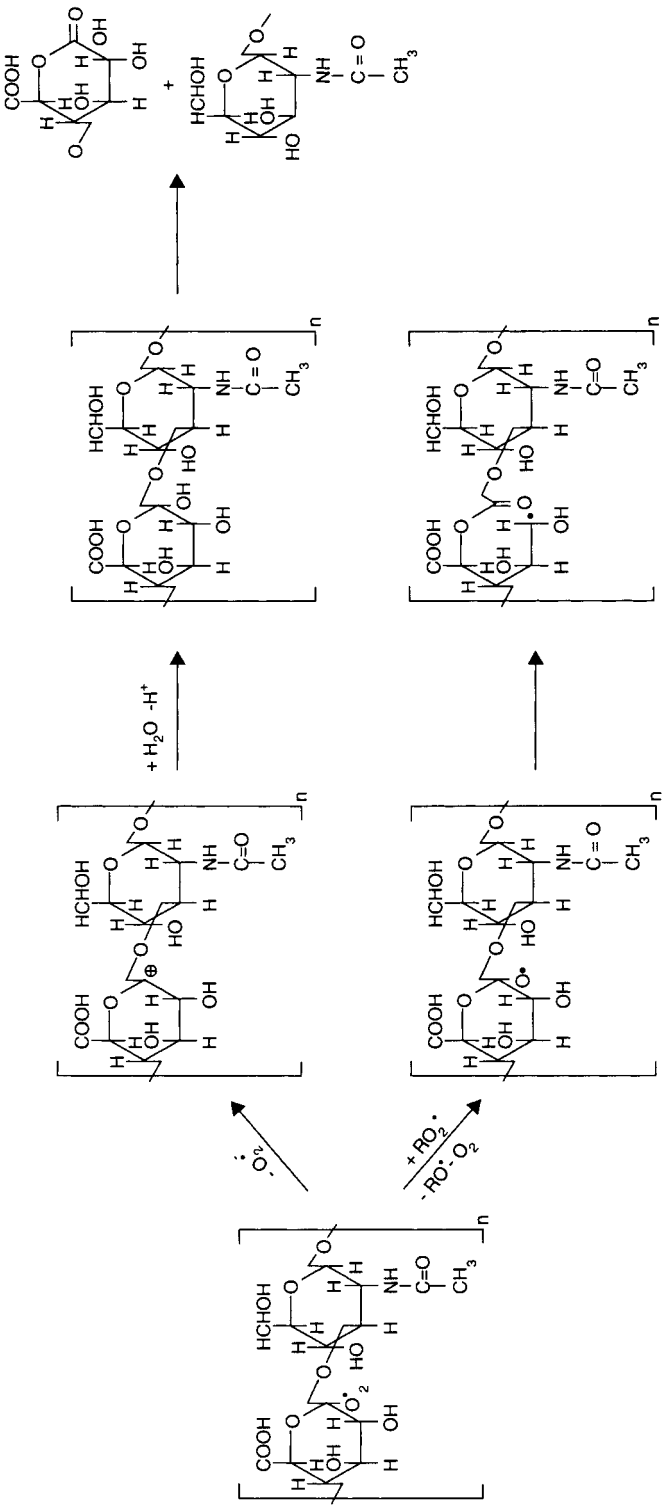
radical. The reaction between HO$^{\bullet}$ and even simple carbohydrates such as glucose is very complex and not yet completely understood because there are many possible rearrangements of the initial products. A summary of these reactions is provided in [62]. The second order rate constant of the initial reaction between HA and HO$^{\bullet}$ radicals is about one order of magnitude ($k \approx 7 \times 10^8$ l mol^{-1} s^{-1}) smaller in comparison to glucose and other simple monosaccharides [63]. This is due to the high viscosity of high-molar-mass HA solutions that reduce the diffusivity of HO$^{\bullet}$ radicals according to the *Stokes-Einstein* equation.

The glycosidic linkage between D-glucuronic acid and *N*-acetyl-D-glucosamine repeating unit of HA is one preferred reaction site of HO$^{\bullet}$ radicals leading to HA degradation under retention of the structure of the monosaccharides. It was shown that the preference of the glycosidic linkage particularly holds if the γ-irradiation is performed in the solid state [64]. The reaction mechanism between HA and HO$^{\bullet}$ radicals can be explained as follows:

1. Abstraction of one hydrogen radical from a (widely unspecific) C-H group under formation of the corresponding alkyl radical. The H$^{\bullet}$ abstraction occurs nearly randomly and there is only a very slight preference for selected positions.
2. Addition of O_2 to the alkyl radical under generation of the corresponding peroxyl radical. The second order rate constant of the O_2 addition is very similar to the H$^{\bullet}$ abstraction, i.e. close to diffusion control.
3. Elimination of $O_2^{\bullet -}$ from the initially generated peroxyl radicals.
4. Cleavage of the oxyl intermediate under generation of the corresponding carbonyl compound. This is one reason why the increase of carbonyl groups is often considered as a marker of "oxidative damage" [54].

The individual steps of the reaction are summarized in Scheme 1.2.

Methods allowing the precise determination of the molar mass were primarily applied to study the HO$^{\bullet}$ radical-induced degradation of HA. For instance, Šoltés and co-workers used rotational viscometry to study the effects of H_2O_2 and Cu^{2+} on HA solutions [65] as well as the inhibitory ("scavenging") effect of selected ibuprofen isomers (ibuprofen is a commonly used analgesic and anti-inflammatory drug). Rotational viscometry is a very sensitive method and enables the detection of even very small changes of the polymer molar mass. In a similar way, the effect of D-penicillamine on HA degradation was also studied [66]. It is shown that the application of D-penicillamine results in two very different effects: there is an initial anti-oxidative action, but this effect is followed by the induction of pro-oxidative effects mediated by an enhanced generation of free radicals. It is assumed that this is a beneficial effect of D-penicillamine because HO$^{\bullet}$ radicals are also strong inhibitors of proteases, which are believed to be responsible for the destruction of joint cartilage under chronic conditions of disease [67] for example.



Scheme 1.2 HO•-radical (generated by γ-irradiation) induced fragmentation of HA. Note that the glycosidic linkage is the preferred reaction site, whereas the remaining C-H groups react rather unspecifically.

SEC (size exclusion chromatography)/MALLS (multi-angle laser light scattering) [68] and HPLC (high performance liquid chromatography) [69], respectively, were also successfully used to study the radical-induced degradation of HA. Finally, NMR (nuclear magnetic resonance) spectroscopy is also a useful tool for analyzing HA degradation, although – in contrast to the above mentioned methods – the detection of the native HA is not possible due to the extreme line-widths high mass compounds are providing. In Figure 1.4, the influence of different γ-radiation doses on the ^{1}H NMR spectra of high-molar-mass HA solution is shown.

Since the NMR method represents a form/concept of a "mobility filter", less mobile, rigid molecules such as high-molar-mass HA are not detectable at all [70]. In contrast, NMR detects compounds more sensitively, the lower their molar mass is. The *N*-acetyl side chain of HA (at about 2.04 ppm) is a very good marker because this side group exhibits a relatively high mobility as it is not entrapped in the rigid carbohydrate ring system. It is evident that the intensity of this resonance increases upon γ-irradiation due to the induced HA degradation. However, it is also doubtless that the *N*-acetyl intensity decreases if very high γ-doses are applied and formate at 8.44 ppm is increasingly detectable. The formate is a well-known final product of the radiolysis of carbohydrates [62]. Therefore, it is evident that at high doses – in addition to the reduction of the molar mass – fragmentations of the pyranose ring systems also occur [70].

The NMR method is not only applicable for the characterization of the solutions of isolated HA, but also for the analysis of human body fluids, e.g. the synovial fluids from patients suffering from RA [71]: subsequently to γ-irradiation, increased *N*-acetyl intensities could be monitored [72]. Concomitantly, the intensity of formate at 8.44 ppm increased. It is one considerable advantage of NMR that both, high- and low-molar-mass compounds can be simultaneously detected. Of course, NMR also offers another additional advantage that even completely unexpected metabolites can be monitored [73]. The contribution of

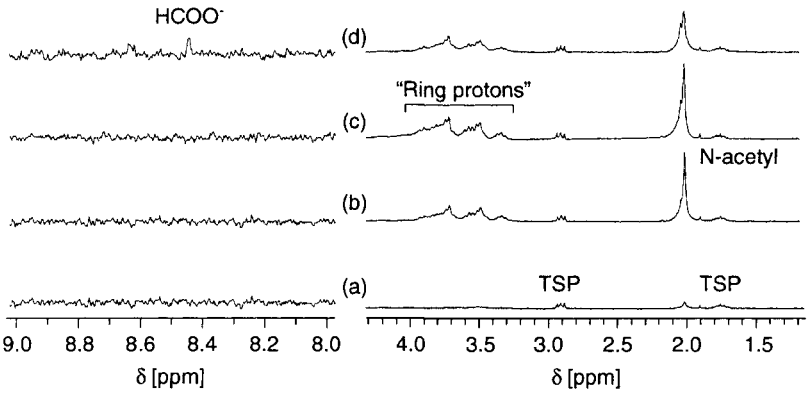


Figure 1.4 ^{1}H NMR spectra of aqueous solutions of HA after exposition to irradiation from a ^{60}Co source. (a) no irradiation, (b) 5.15 kGy, (c) 10.3 kGy and (d) 20.6 kGy.
Abbreviations: TSP, Trimethylsilyl-propionate (standard). Reprinted with (slight modification) permission from [70].

HO• radicals to inflammatory processes in synovial fluids from patients with RA has been recently proven by ESR (electron spin resonance) as well as using spin traps such as 5,5-dimethyl-1-pyrroline-*N*-oxide to convert the highly reactive, short-lived HO• radicals into a more stable, more simple detectable compound [74].

Some authors use a completely different approach: HA or its fragments were not directly detected, but the competition between HA and another (artificially added) compound for potential deactivating of HO• radicals was used as the measure of reactivity. Among other methods, luminol-amplified chemiluminescence was applied [75]: luminol (5-amino-2,3-dihydro-phthalazine-1,4-dione) reacts with HO• radicals under the emission of light. When luminol is present in excess over the generated radicals, the intensity of this light emission depends directly on the number of *in situ* generated radicals. When HA is added to the system, HO• radicals are partially consumed by the reaction with HA. If the second order rate constant for the reaction between HO• and luminol is known, the reactivity of the HA can be calculated by using a *Stern-Vollmer* plot [76]. The reactivities of different ROS with HA as well as with several other GAGs have been recently compared [77].

1.5.2 Reaction of HA with HOCl

HOCl is a molecular agent with strongly different reactivities. It exists as free acid as well as a salt and the ratio between HOCl and NaOCl is strongly pH-dependent [78]. Thus, the HOCl/NaOCl ratio can be easily altered by changing the pH value. It has been shown that there is a very gradual reactivity order if simple amino acids are considered [54]: cysteine or methionine are the most reactive amino acids due to their -SH or -S-S- groups and the second order rate constants of the reactions with HOCl are of the order of 10^7 M^{-1} s^{-1}. An amino group exhibits much lower reactivity ($k \approx 10^4$ M^{-1} s^{-1}), whereas other functional groups are nearly inert against HOCl. Accordingly, in the repeating unit of HA, the glucosamine moiety represents the most reactive site [73].

This was proven by measuring the HOCl/NaOCl consumption by different isolated monosaccharides [79,80]. As time-dependent data are obtained, the relative second order rate constants can be easily calculated. As resulted from one earlier study, viscometry in combination with gel chromatography was used to evaluate (a) the effects of the reagent HOCl and (b) effects of the complete myeloperoxidase (MPO)/H_2O_2/Cl^- system on HA solutions [81]. It was shown that even very small HOCl concentrations (in the μM range) led to a considerable reduction of the HA viscosity, whereas much higher concentrations of HOCl were required to induce the polymer fragmentation. This discrepancy was explained by structural changes in the HA polymer matrix that do already occur in the presence of very small amounts of HOCl [81].

Using ^1H NMR spectroscopy [82], and, a few years later ^{13}C [79] NMR as well, it is evident that *N*-chloroamides are the prime products of the reaction between HA and HOCl. However, *N*-chloroamide is a transient product that decomposes under the generation of acetate, i.e. a cleavage of the *N*-acetyl side

chain occurs. This is an interesting result because the generation of acetate and formate enables the differentiation between the effects induced by HOCl and HO• radicals, respectively. It was also shown that the acetate content is a potent marker of the MPO activity in the synovial fluid from patients with RA [83] and a close correlation between the corresponding NMR peak intensities and the MPO activity could be established.

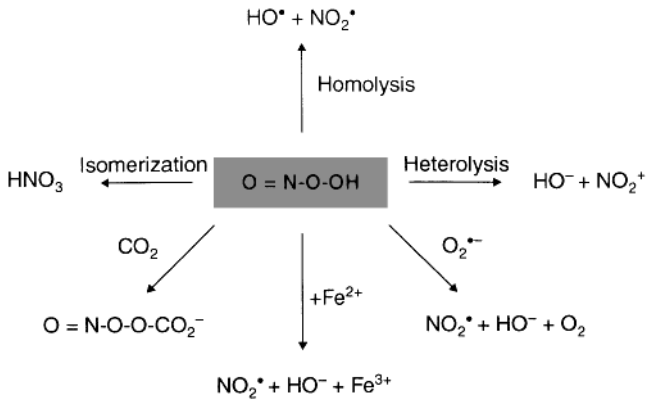
The generation of the HA-derived *N*-chloroamide was later also confirmed by ESR [80]: it was shown that this initial product is converted into an *N*-centered radical that isomerizes into a *C*-centered radical located in the pyranose ring. This is the initial event for the reduction of molar mass of the HA polymer. A few years later, these results were extended by the same authors [84]: it was shown that the initially generated *N*-centered radicals undergo rapid intramolecular abstraction reactions to give *C*-centered radicals at the C-2 position on the *N*-acetylglucosamine rings (*via* 1,2-hydrogen atom shift), and at the C-4 position on the neighboring uronic acid residues (*via* 1,5-hydrogen atom shifts). The C-4-centered radicals, and analogous species derived from model glycosides, undergo pH-independent β-scission reactions that result in a glycosidic bond cleavage [84].

The stability of the initially generated HA-derived *N*-chloroamide has been, so far, controversially discussed. On the one hand, *N*-chloroamides are assumed to be "transient products" [82], and on the other hand, they were described to represent "long-lived species" [85]. This obvious discrepancy might result from the different experimental methods that were used: Schiller *et al.* [82] used the NMR spectroscopy while Rees *et al.*applied the TNB (5-thio-2-nitrobenzoic acid) assay [86] to monitor the stability of *N*-chloroamides. It is not yet clear, whether this assay is capable of differentiating *N*-chloroamides and *N*-chloroamines which are generated by the cleavage of the initially generated *N*-chloroamides. Secondly, the presence of small amounts of transition metals (present as impurities in the used buffers) also affects the stability of *N*-chloroamides.

Another study investigated final products of the HA degradation [87]: it was shown that the NaOCl oxidation of HA yields primarily *meso*-tartaric acid. In contrast, arabinaric acid and glucaric acid are obtained by the oxidation of HA with the *Fenton* reagent. It was suggested that *meso*-tartaric acid represents a useful biomarker of HA oxidation since it is produced by both HOCl and *Fenton* chemistry.

1.5.3 Reaction of HA with Peroxynitrite

Although by far less frequently investigated than HO• radicals or HOCl, peroxynitrite is also capable of degrading HA. However, it is so far unknown if peroxynitrite or one of the products derived thereof are primarily responsible for the observed effects. An overview of the reactive species derived from peroxynitrite is shown in Scheme 1.3. Peroxynitrite-induced effects on HA are similar to the effects by HO• radicals. Thus, it is assumed that peroxynitrite decomposes, under applied experimental conditions, yielding, among other



Scheme 1.3 Potential reactions of peroxynitrite under generation of further (even more) harmful species.

things, HO• radicals [88]. A more detailed investigation using spectroscopic methods as well as MS (mass spectrometry) was recently published [89]: surprisingly, neither NMR nor MS provided any evidence of a peroxynitrite-mediated modification of HA. On the other hand, simultaneously performed ESR experiments gave evidence of C-centered radicals that are most probably generated by the way of HO• radical-like reactivity of peroxynitrite [90]. The reasons for this unequivocal discrepancy are so far unknown.

Although the structures of HA and further GAGs are similar, it must be emphasized that NO• and particularly its derivatives are capable of cleaving heparan, heparan sulfate [91] and chondroitin sulfate [92]. In contrast, however, these ROS are not able to induce fragmentations of HA.

1.6 Hyaluronan Degradation under Inflammatory Conditions

The word "arthritis" (the disease that is the most important one regarding HA) is composed of the Greek words "arth" which refers to joints and "itis" which means inflammation. "Arthritis" is a general term for a group of different diseases that are characterized by the inflammation of one or more joints [54]. What does inflammation mean? From the viewpoint of a physician, the affected patient complains about the so-called "cardinal" indications of inflammation "color, dolor, rubor, and calor", indicating that the affected part of the body is painful, swollen, slightly reddish and feels warm [93].

Besides the activation of proteolytic enzymes, ROS primarily contribute to these symptoms, and they are also generated in many different cell types under stress conditions. For instance, in the inflamed joint, fibroblasts, chondrocytes, macrophages, and especially neutrophilic granulocytes are discussed as the most important sources of ROS [94]. Neutrophils are accumulated in the synovial fluid of the inflamed joints in huge amounts [95], although the prime

reasons for the accumulation and the mechanisms of activation of neutrophils to generate ROS and to release proteolytic enzymes are not yet completely clarified [96]. The increased oxygen consumption by neutrophilic granulocytes upon stimulation under inflammatory conditions is commonly termed "respiratory burst" [97]. A very coarse scheme of the events in the inflamed joint under inflammatory conditions is shown in Figure 1.5.

It is obvious that many various products are generated under these conditions that have different interactions. For instance, ROS generated under these conditions react with functional groups of enzymes that may significantly influence their enzymatic activities. Clearly, as the majority of ROS is generated by the enzymes, this fact also influences the yield of ROS. Thus, evaluation of the detailed role of ROS under physiological conditions is assuredly a rather difficult task.

1.6.1 Generation of ROS under *In Vivo* Conditions

The first step in the generation of all the ROS under *in vivo* conditions is the enzymatic reduction of "normal" oxygen forming $O_2^{\bullet -}$ radicals catalyzed by NADPH oxidase (also termed "respiratory burst oxidase") [99]:

$$2\,O_2 + NADPH \rightarrow 2\,O_2^{\bullet -} + NADP^+ + H^+ \qquad (1.1)$$

Although there are additional pathways of $O_2^{\bullet -}$ generation, this is the most important mechanism under *in vivo* conditions. Please also note that

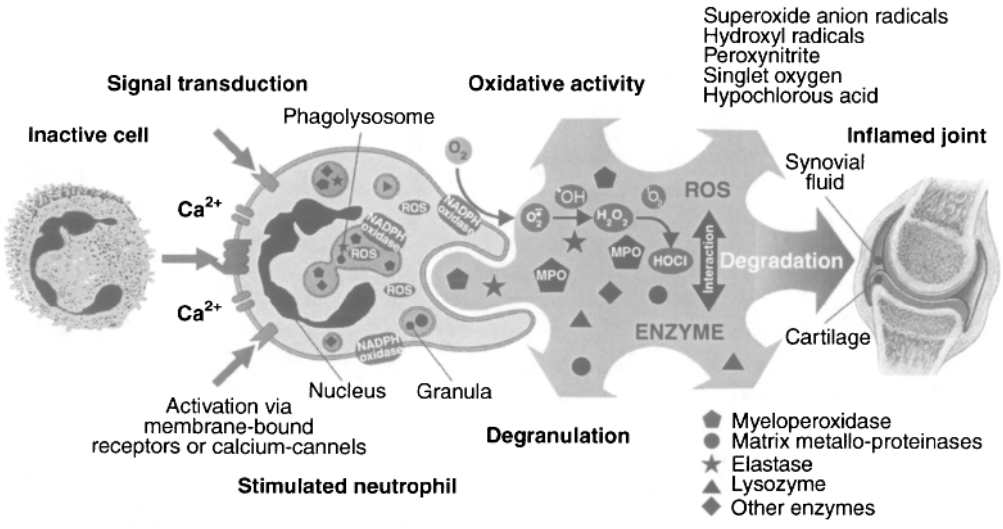


Figure 1.5 Proposed mechanism of cartilage degradation during rheumatic diseases: Neutrophils invade from the blood flow into the joint space. Upon stimulation they release different ROS and proteolytic enzymes. These damage-conferring products lead to the degradation of the high-mass components of articular cartilage under the formation of low-mass components. Reprinted with permission from [98].

$O_2^{\bullet-}$ generation is always accompanied by H_2O_2 generation because $O_2^{\bullet-}$ dismutate either spontaneously or in the presence of superoxide dismutase (SOD) yielding H_2O_2:

$$2\,O_2^{\bullet-} + 2\,H^+ \rightarrow O_2 + H_2O_2 \tag{1.2}$$

As $O_2^{\bullet-}$ and H_2O_2 are simultaneously present, they are often assumed to react with each other (3):

$$H_2O_2 + O_2^{\bullet-} \rightarrow HO^- + HO^{\bullet} + O_2 \tag{1.3}$$

This reaction is the well-known *Haber-Weiss* reaction that is frequently mentioned in a physiological context and highly questioned [100].

Both $O_2^{\bullet-}$ and H_2O_2 are rather slow-reacting species that are not capable of damaging carbohydrates such as HA as already outlined above [54]. These compounds are, however, deleterious in the presence of traces of transition metals, especially Fe^{2+} [69]. Accordingly, H_2O_2 is the starting material for some further ROS formation, including singlet oxygen (4), hypochlorous acid (5) and hydroxyl radicals (6) [101]:

$$H_2O_2 + HOCl \rightarrow {}^1O_2 + H_2O + HCl \tag{1.4}$$

$$H_2O_2 + Cl^- \rightarrow HO^- + HOCl \tag{1.5}$$

$$H_2O_2 + Fe^{2}+ \rightarrow HO^{\bullet} + HO^- + Fe^{3+} \tag{1.6}$$

The last listed reaction (1.6) is the so-called *Fenton* reaction that is already known for more than 100 years but still possesses many mysteries [102]. In short, its biological significance is often indicated to be quite limited because under physiological conditions "free" iron does not exist, but all the iron is firmly bound to protein complexes: in the blood, iron is associated with the protein transferrin and in the cells with the protein ferritin [54].

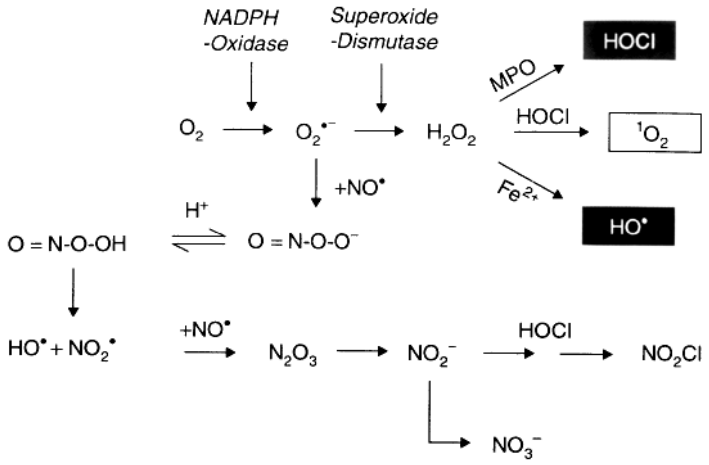
The situation is even more complex, when species such as $NO^{\bullet}$ are additionally considered. $NO^{\bullet}$ and some derived species are analogously termed "reactive nitrogen species" (RNS) [103]. The reader interested in details of these ROS and RNS is referred to the timely review by Šoltés *et al.* [55].

A highly simplified scheme of the generation of physiologically relevant ROS and RNS is shown in Scheme 1.4 although we are focusing here on ROS.

1.6.2 Discussion of ROS Effects under *In Vivo* Conditions

It is extremely difficult to qualify a real role of ROS under *in vivo* conditions due to the following reasons:

1. There is normally a very complex environment comprising, in addition to HA, also a large variety of different proteins, low mass compounds such as amino acids or lipids as well as a variety of



Scheme 1.4 Generation of ROS and RNS under *in vivo* conditions. Note that this is a very simplified summary of potential chemical reactions that does not take into consideration the different locations of enzymes and their substrates. Reactions of HOCl and HO• with HA are emphasized in this chapter.

ions. Therefore, it cannot be expected that the results obtained under *in vitro* conditions with very simple systems can be transferred to the *in vivo* situation.

2. Under *in vivo* conditions, the majority of ROS is generated enzymatically in rather complex metabolic networks. This makes the assessment – which ROS is generated in what amount – very difficult. This particularly holds because there are also many effects of ROS on the related enzyme activities.
3. Finally, there are many antioxidants present in biological tissues that show important effects, not only antioxidative but also prooxidative effects.

1.6.3 Cell-derived Oxidants and Their Effects on HA

Neutrophilic granulocytes cannot be grown as a culture but must be freshly isolated from blood [96]. It is well-known that these cells generate ROS dependently on the conditions of stimulation. To treat HA solutions with the neutrophils and to see which fragments are generated, dependently on the conditions of cell stimulation, seems to be a reasonable approach. Such an experiment has already been performed in 1986 [104]: Using viscometry in combination with HPLC, the authors provided the evidence that there are comparable effects equally if a cell-free superoxide-generating system or a cellular system was used. However, as the results were obtained in the presence of biological buffers, it is questionable if the effects were really caused by superoxide and not by ROS derived thereof. It is, however, also remarkable that no major degradation of HA was observed if only the cell-conditioned media from PMA-(phorbol-myristoyl acetate)-stimulated neutrophils were applied.

Neutrophil-mediated HA degradation was shown to be increased in the presence of the MPO inhibitor azide [104] and is inhibited by dimethylsulfoxide [105] but not by metal chelators such as deferoxamine (DFO) or diethylenetriamine pentaacetic acid (DTPA). Selected specific enzymes are able to prevent the degradation of HA by stimulated neutrophils [106]. Interestingly, the ability to degrade HA seems to be specific to PMA-stimulated PMNs and is not observed with neutrophils stimulated by other compounds such as formyl-methionyl-leucyl-phenylalanine, concanavalin-A or digitonin. This may be related to the increased concentrations of H_2O_2 generated in response to these stimuli, or a greater release of specific enzymes that consume the relevant reactive species [107].

In addition to neutrophils, endothelial cells were also used to degrade a variety of important GAGs. Although these cells were able to degrade heparin, that is very sensitive to a NO-dependent deaminative cleavage, HA is not susceptible to this pathway and thus, it can be concluded that endothelial cells are not capable of generating ROS that lead to the degradation of HA [91].

1.6.4 Synovial Fluids

Synovial fluids (SF) are highly viscous liquids that contain rather high amounts of high-molar-mass HA (normally about 2–3 mg/ml). Due to their profound biological significance, SF have been comprehensively studied. For instance, γ-irradiation of SF results in a dose-dependent degradation of HA [71, 72]. This effect can be effectively suppressed by common free radical quenchers such as *N*-acetyl-L-cysteine [108] and is, thus, mediated by ROS, not by enzymes.

Transition metal-dependent systems such as Fe^{2+}/O_2 or Cu^{+}/O_2 are also able to degrade the SF-present HA, and, it has also been reported that even pure H_2O_2 is able to induce degradation of HA [109]. DFO and mannitol which were indicated to inhibit the degradation of purified HA do not prevent SF degradation, implicating site-specific degradation by metal ions that are not specific on HA. HA is also degraded by xanthine oxidase systems with the inhibitory effects of SOD and catalase, implicating effects of $HO^{\bullet}$ radicals generated from $O_2^{\bullet -}$ in the presence of transition metal ions [110].

1.6.5 Extracellular Matrix

Oxidative damage to the extracellular matrix (particularly to cartilage), and its role in human pathologies has been recently comprehensively reviewed [111]. Although there are many important problems that must still be overcome, the extracellular compartments of most biological tissues are, in fact, significantly less well-protected against oxidative damage than intracellular sites. There is considerable evidence for such compartments being subject to a greater oxidative stress and an altered redox balance. However, with some notable exceptions (e.g. plasma) oxidative damage within these compartments has been neglected and is only poorly understood so far.

In particular, information on the nature and the consequences of the oxidative damage to ECM is lacking despite growing changes in the matrix structure. This can play a key role in the regulation of cellular adhesion, proliferation, migration, and cell signaling. Furthermore, ECM is widely recognized as being a key site of cytokine and growth factor binding. Modification of the matrix structure might be expected to alter such behavior. However, due to the limited available space, no major discussion can be given here and the interested reader is referred to one of the available, excellent reviews [111].

1.7 Interaction of Hyaluronan with Proteins and Inflammatory Mediators

There is increasing evidence that HA, as well as some further GAGs, play an active role in the development of a variety of diseases by their interaction with different cells and proteins [112]. Although the following list should not be expected to provide a complete survey, the aspects outlined below seem to be particularly important:

1. HA is able to interact in an autocrine manner with its cell surface receptors that are located on the same cell, i.e. the cell is influenced by a product produced by this cell itself. For instance, it has been recently shown [113] that low-molar-mass HA induces the proteolytic cleavage of CD44 from the surface of tumor cells and promotes tumor cell migration significantly in this manner.
2. HA interacts in a paracrine manner with a variety of ECM molecules on neighboring cells, and, due to the size of the HA polysaccharide, one HA molecule is capable of interacting directly with different cells, whereby one HA molecule is able to bind hundreds of ECM proteins. This interaction seems to be very important for the structure and the assembly of many different tissues, particularly for the development of cancer [114].
3. *De novo* synthesized HA may be secreted from the cell and subsequently interact with different cell surface receptors, such as CD44, the receptor for HA-mediated motility (RHAMM), the HA receptor for endocytosis (HARE) and many others. It has become evident that these processes mediate at least three different, very important physiological processes: signal transduction, formation of pericellular coats, and the receptor-mediated internalization.

A very short overview on the HA-binding proteins is given here, but due to the limited available space this should be regarded as only a very crude survey. The reader particularly interested in these aspects is referred to one of the excellent reviews that have recently appeared [115, 116]. Please note that due to the importance of HA oligosaccharides, HA-degrading enzymes, i.e. hyaluronidases, are now the focus of intense research.

1.7.1 HA Binding Proteins and Receptors

Hyaladherins are a relatively heterogeneous group of proteins with the common property that they are able to bind HA. These hyaladherins can be differentiated according to their localization, i.e. if they occur extra- (e.g. Versican, Aggrecan) or intracellularly (e.g. the CD44 family or RHAMM) or by the sequence of the HA binding site: The majority of the so far known HA binding proteins and receptors share a common 100 amino acid globular binding domain that is commonly called the "link module" and was first described in the context of the proteoglycans from articular cartilage that also contains a "link protein" mediating the interaction between a HA strand and the GAG. The link module region comprises two different regions: (a) an immunoglobulin domain and (b) two adjacent link modules. Nowadays it is widely accepted that (a) primarily mediates the link protein-proteoglycan interaction, whereas (b) mediate the binding to HA [117].

1.7.2 HA Receptors – Cellular Hyaladherins

Although other HA receptors are also known, we focus here exclusively on the most important ones, namely CD44 and RHAMM. CD44 is a very central molecule and seems to be involved in the development of many various diseases, mainly but not exclusively, different types of cancer. Although there are different isoforms that show different affinities to HA [118], CD44 is basically a transmembrane glycoprotein. Only a small part of the distal extracellular domain seems to be responsible for the ability to bind HA. Mutations of this region significantly decrease HA binding. The binding between CD44 and HA has been elucidated among other methods by means of crystallography and NMR spectroscopy [119]. The crystallography revealed that the CD44 interaction with HA is dominated by shape as well as hydrogen-bonding and identified two conformational forms of the receptor that differ from each other regarding the orientation of a crucial hyaluronan-binding residue (Arg45, equivalent to Arg41 in human CD44). Investigations by multi-dimensional NMR indicated that a conformational transition is induced by the HA binding. This seems a very important mechanism of CD44 regulation.

It must be explicitly stated that the HA binding is not specific at all and the CD44 interacts with many other molecules (often molecules that are, *per se*, capable of interacting with carbohydrates) such as osteoponin [120] or different matrix metalloproteinases [114]. CD44 is up-regulated by pro-inflammatory cytokines such as IL-1 and growth factors such as TGF-β or bone morphogenic protein (BMP-7). HA-CD44 interactions participate in a large variety of cellular functions and these interactions have been summarized by Girish and Kemparaju [112].

Due to this significant importance, there were many attempts to influence HA-protein interactions: firstly, the overexpression of soluble hyaladherins that may displace HA completely from its endogeneous cell surface receptors [121]. Secondly, the administration of defined HA oligosaccharides that

compete with high-molar-mass HA for the binding site resulting in a replacement of the high affinity, polyvalent interaction by a low affinity, low-valency interaction. This strategy leads, for instance, to the inhibition of the *in vivo* growth, local invasion and metastasis of melanoma cells [122]. Although the presence of smaller HA oligomers under *in vivo* conditions is still rather speculative, it has been recently shown that HA fragments can be detected in a sterile UV-B induced inflammation under *in vivo* conditions [123]. Thirdly, the treatment with antibodies that block HA-CD44 interactions, leads to the inhibition of tumor growth and invasion [124].

RHAMM is a HA receptor that can be present either on the cell surface, in the cytoplasm or even in the nucleus. Interactions between RHAMM and HA may trigger a lot of cellular signaling pathways, including those that involve protein kinase C, MAP kinases, phosphatidylinositol and tyrosine kinases [125]. Several recent studies have clearly demonstrated the involvement of RHAMM in the locomotion of TGF-β-stimulated fibroblasts, smooth muscle cells, macrophages as well as Ras-transformed fibroblasts [126], i.e. the processes that are most likely important in the development of cancer.

1.7.3 Extracellular Hyaladherins

These comprise a group of HA-binding proteoglycans that include, for instance, aggrecan and brevican. PGs are the components of ECM and show a characteristic distribution with versican present in different soft tissues, aggrecan predominately in the cartilage, while neurocan and brevican are primarily located in central nervous nerve tissues. We focus here exclusively on the study of cartilage tissue. HA stabilizes the ECM structure through its interaction with several matrix hyaladherins. The most important cartilage PG component, aggrecan, interacts strongly with HA through the HA-binding domain (link module). The binding of PGs to HA is largely mediated by the link protein. The attempts to understand such interactions are being currently intensively investigated [127]. However, it seems clear that such processes are also involved in the pathogenesis of cartilage-affecting diseases.

Finally, it is of interest to note that in addition to HA, other GAGs seem to also be involved in signal transduction events in the extracellular matrix. Shortly, a "GAG code" is coming into consideration [128] that is highly important in the tissue organization as well as in the development of diseases. Thus, many further related investigations may be expected in the future.

1.8 Hyaluronan and Its Derivatives in Use

The most distinctive property of HA is its viscoelasticity in the hydrated state which is able to vary with the rate of shear or oscillatory movement. In fact, the viscosity of a 1% HA solution of $(3–4)\times10^6$ Da, is about 500000 times more than that of water at low shear rate, but can drop 1000-fold when forced through a fine needle. As a consequence, rapid movement reduces HA viscosity, and also

increases the elasticity, which stores energy and permits recovery from deformation [8]. The anomalous viscosity of HA solutions suggests that it should be an ideal biological lubricant, at least by reducing the work load during rapid movements. Indeed, the lubricant role of HA in the soft tissue lining of joints is well established [129], but its contribution to the stiff mass-bearing cartilaginous surfaces is less clear. Finally, both viscosity and elasticity properties are positively related, in a complex way, to molar mass and the concentration, a point that must be considered in the HA surgical and medical uses.

1.8.1 Viscosurgery

The main requirements for a solution to be used intraocularly at surgical interventions can be classified as follows: a) high viscosity at low shear rate to maintain space and manipulate tissues; b) moderate viscosity at medium shear rates to allow easy manipulation of surgical instruments and intraocular lenses within the polymer solution; c) very low viscosity at high shear rates to minimize the pressure needed to expel the solution through a thin cannula; d) high degree of elasticity to protect intraocular tissues, especially the endothelial cells of the cornea, from contact with surgical instruments; e) the pH and osmolality within the ranges 6.5–8.5 and 200–400 mOsm.

At the present, several companies are marketing various HA solutions, which compete for the attention of eye surgeons, and today the extraction and/or replacement of a damaged lens can be classified as a routine ophthalmologic intervention. Damage to ocular tissues might be hereditary-based or even occur during different pathophysiological events. For example, cataracts account for approximately 42% of all blindnesses and, due to the alarming prevalence of *Diabetes mellitus* worldwide, the diabetic cataract attracts ever-increasing attention [130, 131]. Today HA [Healon® (sodium hyaluronate), Pharmacia, Uppsala, Sweden] is used as a soft surgical instrument for cataract extraction, intraocular lens implantation, keratoplasts, glaucoma surgery, trauma and posterior segment surgery throughout the world.

1.8.2 Viscoprotection

The highly viscoelastic HA available for therapeutic purposes, has also proved to be very efficient in alleviating discomfort in "dry eye syndrome". Although HA is not present in tears, in many aspects sodium hyaluronate is similar to mucin, a major component of tears. Mucin with a mean-molar-mass of about 2 MDa exhibits, similarly to HA, typical viscoelastic and shear-thinning behavior. This glycoprotein plays an important role in the lubricating, cleansing, and water-retaining properties of tears. The usefulness of a HA solution as a tear substitute resides in its water-entrapment capacity (hydration) and its function as a viscoelastic barrier between the corneal and conjunctival epithelia and noxious environmental factors (dust, smoke, etc.) [132]. During eye blinking, the HA eye drops are elastically deformed but not removed from the surface of the eye due to blinking movements.

1.8.3 Viscosupplementation

Osteoarthritis is one of the most common forms of arthritic diseases. It affects more than 10% of the world population. OA occurrence is age-progressive and, if not cured, it may result in severe disabilities. It has been claimed that 50% of people over 65 have evidence of OA of the knee, and nearly 100% of the population at the age of more than 75 have diagnosed changes in at least one joint [17].

The idea of intra-articular application of HA has been supported by the fact that the synovial fluid in OA joints lacks sufficient shock absorption and lubrication properties mostly due to the presence of HA of low mean-molar-mass, as a consequence of an absence of viscous high-molar-mass HA [133]. Thus, the so called "viscosupplementation", a series of HA injections, has been designed to change the character of the joint fluid. Although the mechanism of the affection of HA injection(s) applied into the OA knee joints is not yet fully established [17], it is claimed that the viscosupplemented high-molar-mass HA increases the joint fluid viscoelasticity, stimulates the production of endogenous HA, inhibits the effects of inflammatory mediators, decreases cartilage degradation, and promotes cartilage matrix synthesis. Currently, HA injections (sodium salts) are approved for the treatment of OA in those patients who have persistent pains or are unable to tolerate conservative treatment or joint replacement. Viscosupplementation for other joints (e.g. shoulder) is currently being investigated.

1.8.4 Vehicle for the Localized Delivery of Drugs to the Skin

HA has also been investigated as a drug delivery agent for various routes of administration, including ophthalmic, nasal, pulmonary, parenteral, and topical. In fact, regulatory approval in the USA, Canada, and Europe has been granted recently for 3% diclophenac in 2.5% HA gel, Solaraze®, for the topical treatment of actinic keratoses, which is the third most common skin complaint in the USA [134]. The gel is well tolerated, safe and efficacious and provides an attractive, cost-effective alternative to cryoablation, curettage or dermabrasion, or treatment with 5-fluorouracil.

1.8.5 Electrospinning for Regenerative Medicine

Electrospinning techniques enable the production of continuous fibers with dimensions on the scale of nanometers from a wide range of natural and synthetic polymers [135]. The number of recent studies regarding electrospun polysaccharides and their derivatives, which are potentially useful for regenerative medicine, is dramatically increasing.

As a major component of the natural extracellular matrix, HA has also attracted considerable attention in electrospinning. However, it is very difficult to electrospin an aqueous HA solution because its unusual high viscosity and surface tension both hinder the electrospinning process. In addition, the

strong water retention ability of HA leads to the fusion of electrospun nanofibers on the collector due to the insufficient evaporation of the solvent during electrospinning. The fabrication of HA into nanofibrous membranes from aqueous solution was successfully carried out only after the development of blowing-assisted electrospinning (electro-blowing system) [136]. HA nanofibers were fabricated using a dimethylformamide (DMF)/water mixture, and the use of DMF significantly decreased the surface tension without changing the viscosity of the HA solution. HA/gelatin nanofibrous matrices can also be produced by this method.

HA-based nanofibrous membranes have been extremely attractive as biomimetic tissue engineering scaffolds and wound healing materials. In order to mimic the architecture of the natural ECM using electrospinning, a thiolated-HA derivative was synthesized and electrospun to form nanofibrous matrices [137].

1.9 Concluding Remarks

It could be hard to find such a unique biopolymer that exhibits the many excellent properties of hyaluronan/hyaluronic acid. Hyaluronan is, from a commercial viewpoint, a remarkably valuable biopolymer whose production is still growing through microbial fermentation. The research development has been focused on improving the key quality parameters – purity and molar mass. Having made exceptional progress over the last decade, metabolic engineering tools for hyaluronan production represent novel engineering challenges. Reflecting a variety of natural functions, hyaluronan has found a number of applications in, for example, medicine, cosmetics, and biotechnology. The investigation into the important role which hyaluronan plays in biological systems has recently led to numerous publication activities meeting all aspects of physiological and pathological areas of interest.

An enormous challenge still remains to reduce disease-derived human suffering if a biological specificity against deleterious actions of oxidants in living systems is better understood. The management of the pathogenesis of various diseases may eventually lead to the discovery of novel therapeutic and clinical strategies.

Acknowledgements

The author (J. S.) wishes to thank all colleagues and friends who helped in writing this review. This work was supported by the German Research Council (DFG Schi 476/7-1 as well as TR 67, project A2). The work was financially supported, partly, by the VEGA grants project Nos.: 2/0056/10 and 2/0011/11 of the Slovak Academy of Sciences.

The authors are indebted to Eriko Hagiya, who is in charge of the Glycoforum website http://www.glycoforum.gr.jp managed by Seikagaku Corporation and Mizutani Foundation, for permitting the reprint of figures 1.2 and 1.3.

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2

Polysaccharide Graft Copolymers – Synthesis, Properties and Applications

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Abstract

Natural polysaccharides have received more attention due to their advantage over synthetic polymers, such as their being non-toxic, biodegradable and low cost. Modification of polysaccharides through graft copolymerization improves the properties of natural polysaccharides. Polysaccharide-based graft copolymers are of great importance and widely used in various fields. Graft copolymers play an important role as reinforcing agents in the preparation of green composites. These graft copolymers when subjected to studies on composting and soil-burial biodegradation were found to be biodegradable in nature.

Modification of natural polysaccharides through various graft copolymerization techniques is discussed in this chapter. Characterization of graft copolymers using different techniques like FT-IR, ^{13}C-NMR, SEM, XRD, TGA, DTA and DTG along with their physical, chemical and mechanical properties are discussed as a function of different reaction conditions of their synthesis. Applications for modified polysaccharides include drug delivery devices, controlled release of fungicides, selective water absorption from oil-water emulsions and purification of water.

Keywords: Polysaccharides, green composites, graft copolymers, drug-fungicide delivery, thermal stability, biodegradation

2.1 Introduction

Recently modified polysaccharides have become a major area of scientific research. The renewed interest in polysaccharides has resulted in a great revolution, and such biodegradable materials have become on par with, and even superior to, synthetic materials. The re-emergence in the use of natural materials as reinforcing agents has taken place in, for example, automobiles, packaging and building materials. Natural fibers and their derivatives have been found to be better reinforcing materials for the preparation of composites due to their ecological friendliness and attractive mechanical properties [1–8].

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (35–58)

Polysaccharides and their graft copolymers find extensive applications in diversified fields. Grafting is known to improve the characteristic properties of backbones. Such properties include water repellancy, thermal stability, flame resistance, dyeability and resistance towards acid-base attack and abrasion. Modification of natural cellulosics through graft copolymerization of methyl methacrylate onto *Cannabis indica*, rayon, jute, cotton, etc., has resulted in the improvement of their morphology and other physicochemical properties [9–23].

Polysaccharides possessing the property of swelling in water or biological fluids can be converted to superabsorbents through graft copolymerization with different hydrophilic monomers and crosslinking them with different types of crosslinkers like N,N'-methylene-bis-acrylamide, hexamethylene tetramine, hexamethylene diamine, etc. The graft copolymerized and crosslinked polysaccharides are cost effective, biodegradable and quite efficient when used in various technological processes. They provide a better option for the artificial synthetic materials. The end products obtained with improved properties can be used in various fields such as sustained drug delivery systems, controlled release of insecticides and pesticides to plants in agricultural and horticultural practices, release of water to plants during draught conditions, water treatment and membrane technology [24–27].

2.2 Modification of Polysaccharides through Graft Copolymerization

Polysaccharides can be modified through graft copolymerization using different techniques such as chemical and radiation techniques [28–33].

Graft copolymerization is one of the most common tools in the hands of chemists to bring-out the desired changes in the backbone polymer. Different vinyl monomers using chemical initiators or radiations can be grafted onto backbone thereby incorporating properties such as water repellency, water absorbency, acid-base resistance, thermal stability and dyeability in the backbone polymers [34–37].

2.2.1 Graft Copolymerization Using Chemical Initiators

Different types of chemical initiators such as FAS-KPS, benzoyl peroxide, azo-bis-iso-butylnitrile, H_2O_2, ascorbic acid-KPS, ammonium persulphate, ceric ammonium persulphate, ceric ammonium nitrate, etc., can be used for the graft copolymerization of various vinyl monomers such as methylacrylate, methyl methacrylate, acrylic acid, methacrylic acid, ethyl methacrylate, vinyl acetate, acrylamide, etc., onto polysaccharides [38–40]. Figure 2.1 shows the proposed mechanism through which the grafting can be explained [39].

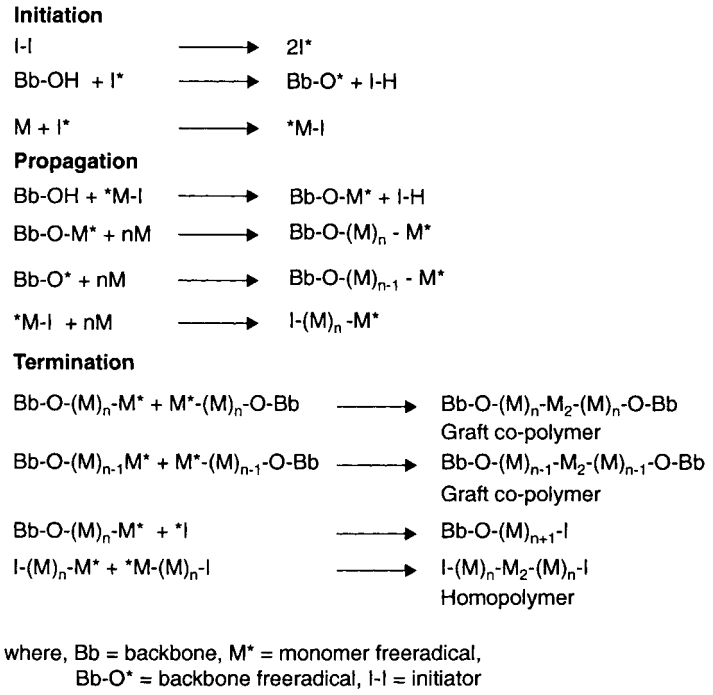


Figure 2.1 Proposed mechanism of grafting using chemical initiators [39].

However, if the grafting is carried out in the presence of ceric ion, it forms a complex with the polysaccharide:

$$Ce^{4+} + \text{PolyS-}CH_2OH \rightarrow \text{Complex} \rightarrow Ce^{3+} + H^+ + \text{PolyS-}CH^*\text{-}OH \quad (2.1)$$

where, PolyS-CH_2OH = Polysaccharide backbone

In order to minimize the homopolymerization and chain transfer reactions, ceric ion initiated graft copolymerization reactions are usually carried out at lower temperatures. Ceric ion initiated reaction is highly specific in nature.

During grafting using ceric ion as an initiator, the presence of nitric acid is found to play an important role. Ceric ion in water reacts in the following manner:

$$Ce^{4+} + H_2O \rightarrow \left|Ce(OH)_3\right]^{3+} \quad (2.2)$$

$$2\left|Ce(OH)_3\right]^{3+} \rightarrow \left|Ce\text{-}O\text{-}Ce\right]^{6+} + H_2O \quad (2.3)$$

Thus, in water as the reaction medium, ceric ion exists as Ce^{4+}, $[Ce(OH)_3]^{3+}$ and $[Ce\text{-}O\text{-}Ce]^{6+}$. No grafting was found in the absence of nitric acid as

[Ce-O-Ce]$^{6+}$ since its larger size is unable to form complex with the backbone. However, with an increase in [HNO_3], the equillibria (Equations 2.2 and 2.3) shifts towards more and more of Ce^{4+} and [$Ce(OH)_3$]$^{3+}$, which can easily form complex with the functional groups of backbone. The complex on decomposition gives rise to free radical sites on backbone (Equation 2.1) where grafting takes place (Equation 2.4):

$$\text{PolyS-CH}^{*}\text{-OH} + {}^{*}\text{Mn} \rightarrow \text{PolyS-CH}\begin{matrix}\vert \\ (\text{M})_n \\ \vert \\ \text{OH}\end{matrix} \qquad (2.4)$$

Graft Copolymer

2.2.2 Graft Copolymerization Using Radiations as Initiators

Different types of radiations can be used for the graft copolymerization of vinyl monomers onto polysaccharides, e.g., γ-radiations and microwave irradiation methods [22–24]. Initiation of grafting using microwave radiations is explained below:

Initiation

$$\text{M} + \text{MW} \rightarrow \text{M}^{*} \qquad (2.5)$$

$$\text{M} + {}^{*}\text{OH} \rightarrow {}^{*}\text{M-OH} \qquad (2.6)$$

$$\text{Bb} + \text{MW} \rightarrow \text{Bb}^{*} \qquad (2.7)$$

where, MW = microwave radiation.

In the case of γ-radiations, induced grafting initiation can be presented through the following equations:

Initiation

$$\text{nM} \xrightarrow{\gamma\text{-Radiations}} {}^{*}\text{M}_n \qquad (2.8)$$

$$\text{H}_2\text{O} \xrightarrow{\gamma\text{-Radiations}} \text{H}^{*} + {}^{*}\text{OH} \qquad (2.9)$$

$$\text{Bb-OH} \xrightarrow{\gamma\text{-Radiations}} \text{Bb-O}^{*} + \text{H}^{*} \qquad (2.10)$$

$$\text{Bb-OH} + {}^{*}\text{OH} \rightarrow \text{Bb-O}^{*} + \text{H}_2\text{O} \qquad (2.11)$$

$$M + {}^{*}OH \rightarrow {}^{*}M\text{-}OH \tag{2.12}$$

$$Bb\text{-}OH + {}^{*}M\text{-}OH \rightarrow Bb\text{-}O\text{-}M^{*} + H_2O \tag{2.13}$$

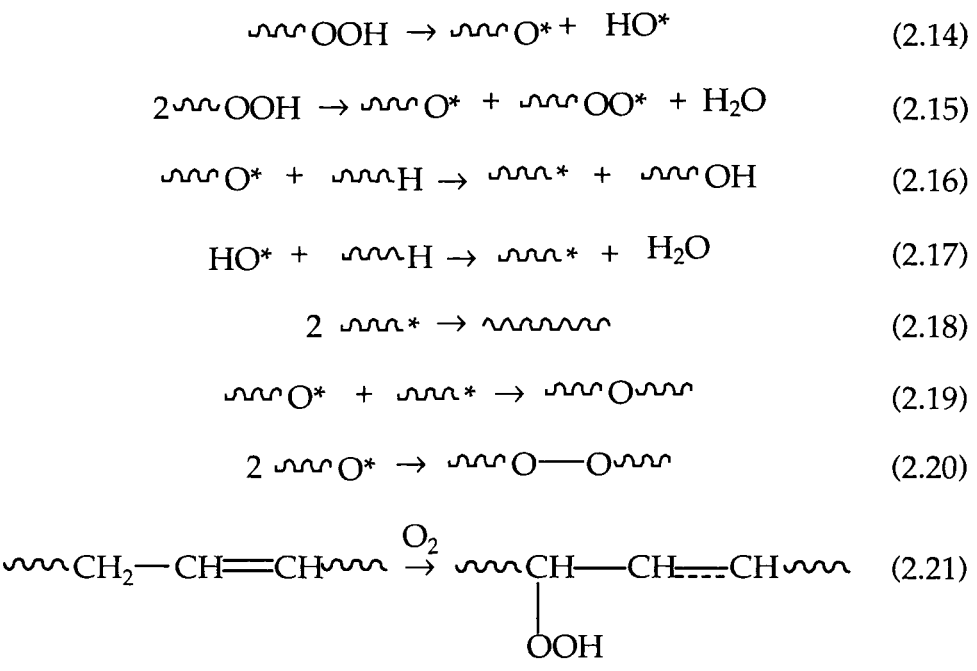
2.3 Different Reaction Conditions for Graft Copolymerization

Grafting of vinyl monomers onto polysaccharides can be carried out under different reaction conditions such as in air, under pressure, under vacuum, under the influence of γ-radiations and microwave radiations.

2.3.1 In Air (IA) Graft Copolymerization

Grafting of different vinyl monomers onto polysaccharides can be done in open air atmosphere. But the graft yield is always lesser than grafting carried out under nitrogen atmosphere due to the interference of atmospheric oxygen [39, 41].

Hydroperoxide linkage decays with the following pathways:



(2.14)

(2.15)

(2.16)

(2.17)

(2.18)

(2.19)

(2.20)

(2.21)

2.3.2 Under Pressure (UP) Graft Copolymerization

Under pressure grafting is one of the techniques for the incorporation of acid-base resistance, water repellancy, water absorbency, thermal stability and other morphological changes in the polysaccharides [42–44]. Under pressure,

there is a maximum chance of collisions of inter-free radical species, thereby resulting in early decay of the propagating free radical chain and giving rise to short chain graft copolymers. Under high pressure, homopolymerization also predominates graft copolymerization, $OH^{\cdot}$ radicals preferably react with the monomer molecules and results in the termination of growing homopolymer chains. Hence, a low graft yield was obtained in the case of grafting under pressure.

$$^{\cdot}OH + M \rightarrow OH - M^{\cdot} \xrightarrow{nM} H - O - (M)^{\cdot}_{\overline{n+1}} \qquad (2.22)$$

$$HO - (M)^{\cdot}_{\overline{n+1}} + {}^{\cdot}(M)_{\overline{n+1}}\,OH \rightarrow \underset{\text{Homopolymer}}{HO - (M)_{\overline{n+1}}\,(M)_{\overline{n+1}}\,OH} \qquad (2.23)$$

Under pressure there are always chances of collision among the free monomer radicals resulting in homopolymerization and short length graft copolymers [34].

2.3.3 Under Vacuum (UV) Graft Copolymerization

In order to avoid the interference of atmospheric oxygen during graft copolymerization of vinyl monomers onto polysaccharides, grafting can be done under vacuum. This will result in different morphological and physico-chemical behaviour of the material along with higher graft yield [45–48].

2.3.4 Graft Copolymerization Under the Influence of γ-Radiations

γ-radiation is the best source as an initiator for carrying out grafting of monomers onto polysaccharides. It is the most effective technique of grafting, and Co-60 can be used as the source of radiations. A clear-cut morphological difference can be seen in the grafted polysaccharides with enhanced thermal, chemical and physical properties [24, 49, 50].

2.3.5 Graft Copolymerization Under the Influence of Microwave Radiations (MW)

In the case of grafting in the presence of microwave radiations, the electromagnetic waves which pass through the reaction medium cause the chain carrier free radicals to oscillate at a high speed, therefore, there exists a rapid collision between the different free radical species, resulting in chain termination reaction.

Under microwave, grafting can be carried out in minimum time, ranging anywhere from a few seconds to five minutes. Advantages with grafting

under microwave influence lie in minimum homopolymer formation. Hence, maximum graft yield can be obtained in a minimum time interval [34]. Moreover, it results in a minimum disturbance in the crystallinity of the backbone [28, 51–53]. However, the graft copolymers obtained are of a short length in nature.

The grafting of ethyl acrylate onto sunn hemp cellulose (SHC) should follow the proposed mechanism given in Figure 2.2. The optimal time of polymerization onto SHF is found to be 40 minutes [54].

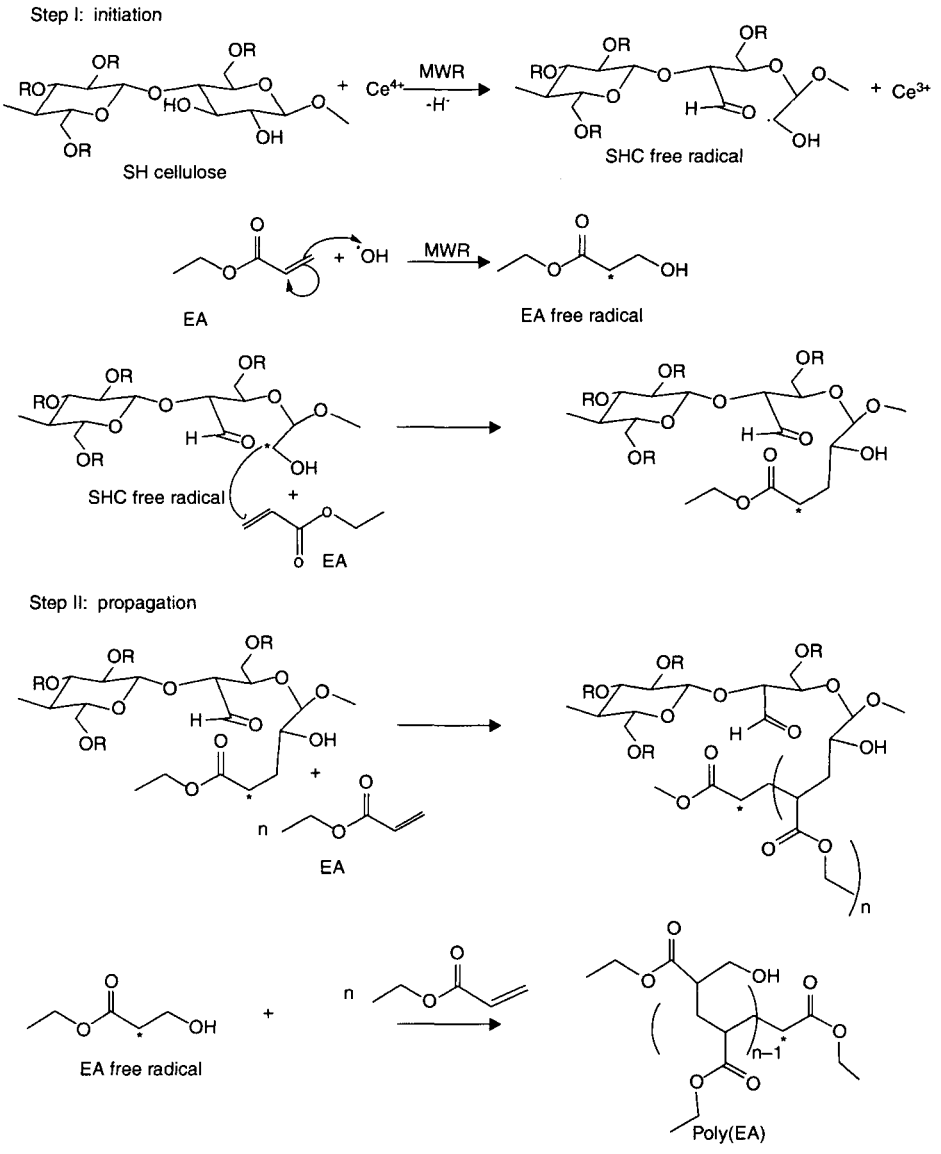


Figure 2.2 Proposed mechanism of grafting of ethyl acrylate onto sunn hemp cellulose (SHC) [54].

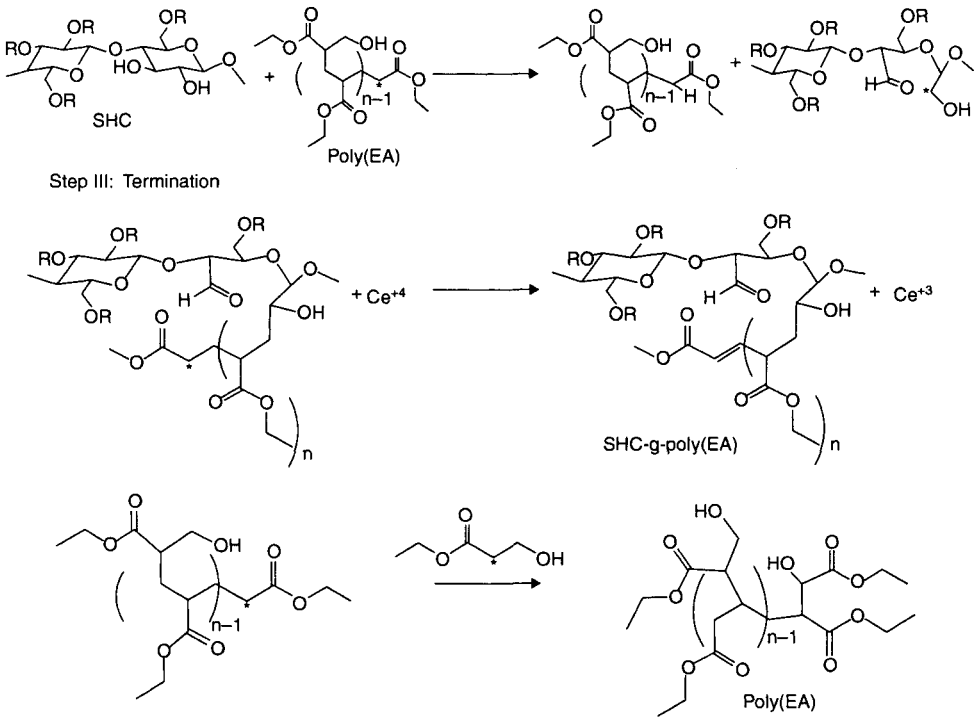


Figure 2.2 (cont.) Proposed mechanism of grafting of ethyl acrylate onto sunn hemp cellulose (SHC) [54].

2.4 Characterization of Graft Copolymers

Characterization of graft copolymers constitutes an important component of graft copolymerization research. It includes different techniques such as FT-IR, ^{13}C-NMR, scanning electron microscopy (SEM) and X-ray diffraction (XRD).

2.4.1 FT-IR

Functional groups of the backbone and their graft copolymers can be characterized through FT-IR technology and a clear cut distinction between the two can be made. This helps in ascertaining that grafting of vinyl monomer has taken place onto the backbone through covalent bonding (Figure 2.3) [55, 56].

2.4.2 ^{13}C NMR

Solid-state ^{13}C NMR of graft copolymers exhibits the signals of carbons corresponding to carbonyl and other functional groups along with methyl and methylene groups in addition to normal peaks for carbon atoms occurring at different positions (Figure 2.4) [57].

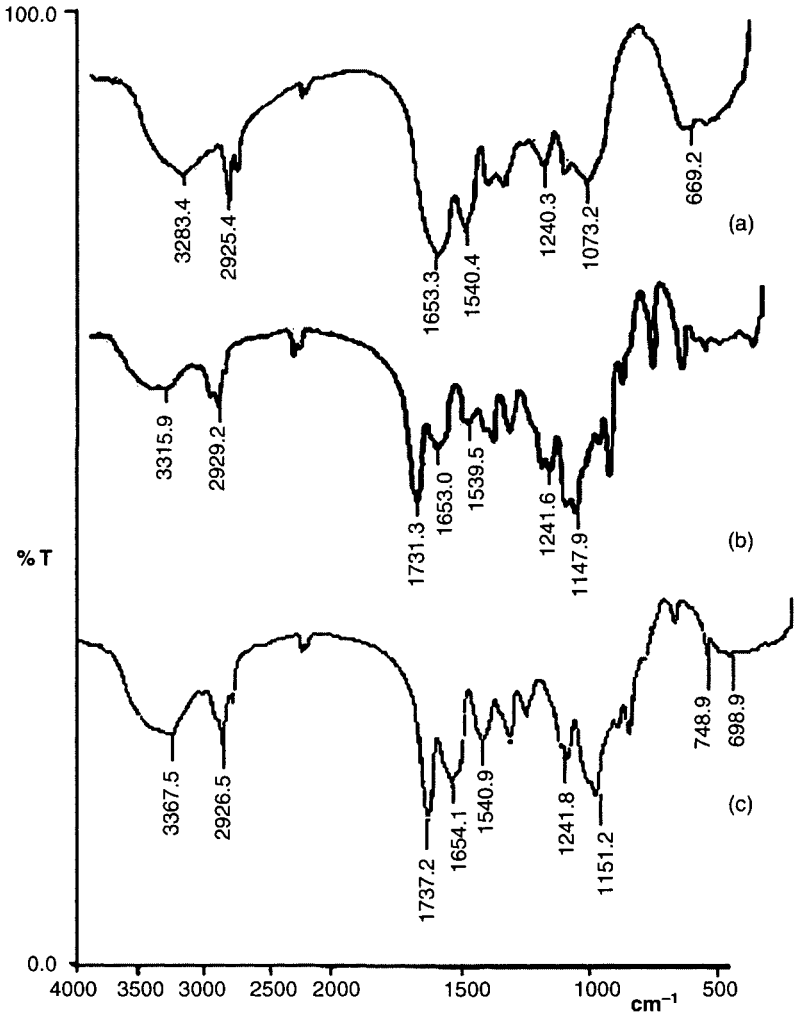


Figure 2.3 FTIR Spectra of (a) SPC (b) graft copolymers-IA, (c) graft copolymer-MW [55, 56].

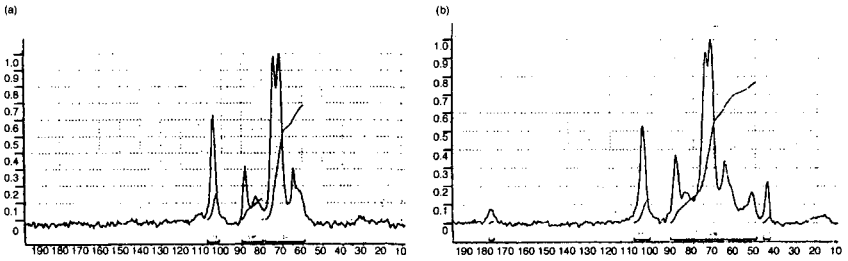


Figure 2.4 Solid-state ^{13}C NMR of (a) raw flax (b) flax-g-poly(MMA) [57].

2.4.3 SEM

Intricacies brought about by the graft copolymerization of different vinyl monomers onto various polysaccharide backbones under different reaction conditions like in air, under pressure, under-vacuum, under the influence of γ-radiations and microwave irradiation using chemical initiators or radiations as the source of initiation, clearly show the morphological changes, which are quite evident through the scanning electron microscopic studies of different graft copolymers (Figure 2.5) [55, 56].

2.4.4 XRD

X-ray analysis is carried out using Cu Kα (1.5418Å) radiations, Ni-filter and scintillation counter at 40 KV and 40 mA on rotation between 13°–25° at 2θ-scale at 1 second step size and increment of 0.01 degree with 0.5 degree or 1.0mm of divergent and anti-scattering slit. Continuous scans are taken and differences are obtained by tapping small size sample with glass slide on PMMA holder.

In the case of cellulosics percentage, crystallinity and crystalline index are calculated as follows [23]:

$$\% \text{ Cr} = \frac{I_{22}}{I_{22} + I_{18}} \times 100 \tag{2.24}$$

$$\text{C.I.} = \frac{I_{22} - I_{18}}{I_{22}} \tag{2.25}$$

where, I_{22} and I_{18} are the intensities of the crystalline and amorphous regions at 2θ-scale close to 22° and 18°, respectively.

XRD of other polysaccharides can be studied and analysed by using the Scherrer equation:

$$L = 0.9\lambda \; / \; \cos\theta \; b \; (\Delta 2 \; \theta b)$$

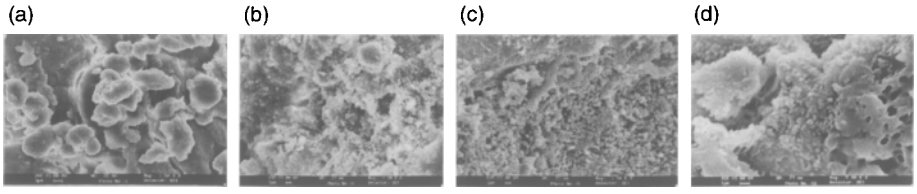


Figure 2.5 SEMs of (a) SPC (b) graft copolymers-IA, (c) graft copolymer –MW (d) graft copolymer-UP [55, 56].

where, L= coherent length, λ = wave length of the radiations, θb = glancing angle and $\Delta 2\ \theta b$ = difference in angle at the end of FWHM.

Most of the cellulosics possess both crystalline and amorphous regions. The occurrence of both types of features in cellulosics and other polysaccharides are the indications of ordered and disordered regions. In the case of polysaccharides, the incorporation of poly(vinyl) chains onto the backbone of the polymer, disturbs its crystalline lattice and causes a marked loss in crystallinity with increase in percentage grafting. Crystalline index gives a quantitative measure of the orientation of the polysaccharide crystals in the backbone. A lower crystalline index in the case of graft copolymers means poor order of arrangement of polysaccharide crystals. This can be due to misorientation of the polysaccharide crystals to the axis after the grafting process (Figure 2.6) [55, 56].

Thus, during grafting, crystallinity decreases rapidly with reduction in its stiffness and hardness. Hence, morphological transformation can be observed on graft copolymerization of vinyl monomers onto polysaccharides of different origins [58].

However, in certain cases polysaccharides have been found to exhibit higher crystalline character on graft copolymerization with vinyl monomers followed by crosslinking [40]. This can be due to strengthening of the crystalline lattice of the backbone and is also dependent upon the chemical nature of the monomers [59–61].

2.4.5 Thermal Studies

Generally polysaccharides and their derivatives are known to degrade by thermo-oxidation, dehydration, depolymerisation and glycosan formation. Furthermore, polysaccharides degrade to smaller units by depolymerisation followed by pyrolysis. Pyrolysis produces the products which can combine to form the residue [62].

Thermogravimetric analysis (TGA) of polysaccharides and their grafted derivatives are carried out as a function of weight loss versus temperature.

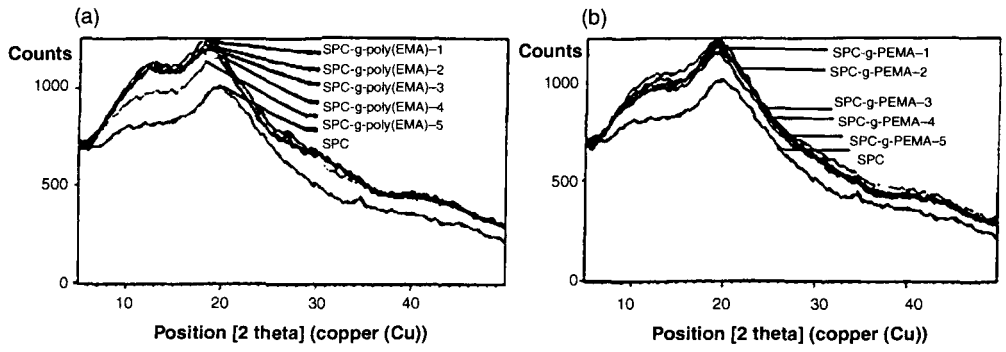


Figure 2.6 XRD of SPC and its graft copolymers (a) in air (b) in microwave [55, 56].

Degradation occurs in various forms such as deacetylation, dehydration, decarboxylation and chain scissions, resulting in thermograms with single phase or two and three phases of decomposition. In the case of graft, the copolymers shift to a higher temperature could be accounted for by the increase in the covalent bonds varying with percentage grafting. In most cases, major weight loss occurs in the first phase due to polysaccharide decomposition while another phase may correspond to the oxidation of char. Grafted backbones are found to possess higher initial and final decomposition temperatures in comparison to backbones [63–66].

In the case of differential thermal analysis (DTA), most of the graft copolymers exhibit exothermic peaks at higher temperatures. These exothermic peaks can be related to different decomposition phases of TGA. Exothermic peaks at a lower temperature correspond to the release of energy during the volatilization process, whereas, higher temperature exothermic peaks correspond to the decompositions of other polysaccharide contents [67].

Differential thermal analysis (DTG) deals with the rate of decomposition of mass with respect to time. In the case of most of the graft copolymers, rate of weight loss as a function of time (mg/min) is lower than that of backbones [68]. However, in some other cases it has been found to be higher due to the disturbance in the crystalline lattice of the natural polysaccharides upon the incorporation of polyvinyl chains through graft copolymerization (Figure 2.7).

2.5 Properties of Polysaccharide Graft Copolymers

Graft copolymerization is the technique that can be used to incorporate desired physical, chemical and mechanical properties in polysaccharides. Moreover, it assists in getting the morphological changes in the end-products.

2.5.1 Physical Properties

2.5.1.1 Moisture Absorbance or Retardance Behaviour

Graft copolymerization can greatly affect the moisture absorbance behaviour of different types of backbones. Depending upon the chemical nature of vinyl monomers used, the end product could be hydrophobic or hydrophilic in nature. Hydrophobic or hydrophilic long chain organic moieties replace the free hydroxyl groups present at C-2, C-3, C-6 and other positions of polysaccharide units, thereby resulting in morphological transformations with change in moisture absorbance or retardance behaviour [69–74] (Figure 2.8).

2.5.1.2 Swelling Behaviour in Different Solvents

Swelling of graft copolymers in different solvents depends on the chemical nature of the solvents. Water affects the changes in the hydrogen bonding of

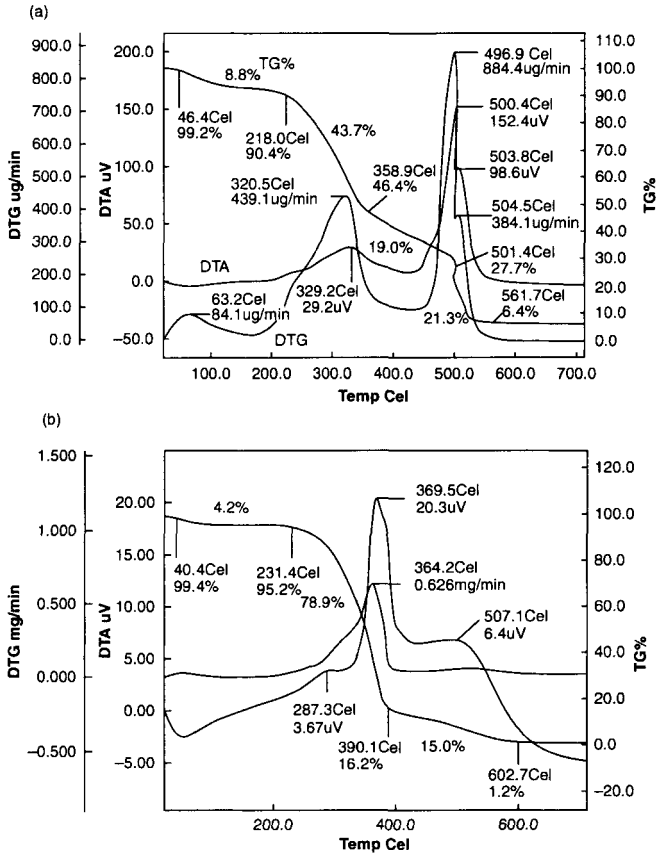


Figure 2.7 TGA/DTA/DTG of (a) SPC and (b) graft copolymers-IA [55, 56].

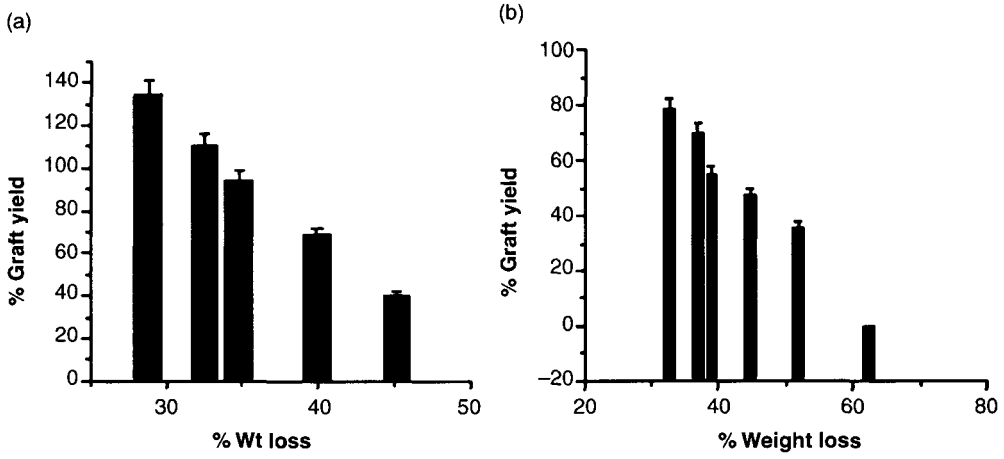


Figure 2.8 Moisture resistance studies of graft copolymers (a) in air (b) in microwave [55, 56].

polysaccharides and penetrates deeper into the matrix. In the case of poly(MA) chains grafted polymers, swelling is more in DMF and lesser in water and n-butanol. This is because there is more chemical affinity of poly(MA) chains for DMF and less affinity towards water and n-butanol. This is similar to the cases with other poly(vinyl) chains' swelling behaviour in different solvents like methanol, n-butanol, DMF and water [75].

2.5.1.3 *Salt Resistant Swelling*

Certain crosslinked graft copolymers of polysaccharides are found to exhibit salt resistant swelling in different concentrations of salts. Swelling decreases with increase in salt concentration and is found to be cationic charge dependent. Depending upon the cations, swelling is found to follow the order: Na^{+}> NH_4^{+}> Mg^{+2}> Ca^{+2}> Ba^{+2}>Fe^{+3}.

Thus, among the same valent ions, the lesser the cation size, the greater is the swelling capacity. Moreover, decreased swelling capacity with increased cation charges of the salts, is due to the charge screening effect of additional cations causing non-perfect anion-anion electrostatic repulsion, leading to decreased osmotic pressure (ionic pressure), i.e., the difference between polymer network and the external solution [76, 77].

2.5.2 Chemical Properties

2.5.2.1 *Acid Resistance*

Graft copolymers have been found to show higher acid resistance in comparison to natural polysaccharides. This increase in acid resistance is due to the incorporation of poly(vinyl) chains onto vulnerable sites like –OH and CH_2OH groups [60, 78, 79] (Figure 2.9).

2.5.2.2 *Base Resistance*

Incorporation of hydrophobic poly(vinyl) moieties through graft copolymerization onto natural polysaccharides results in the decrease of base sensitivity

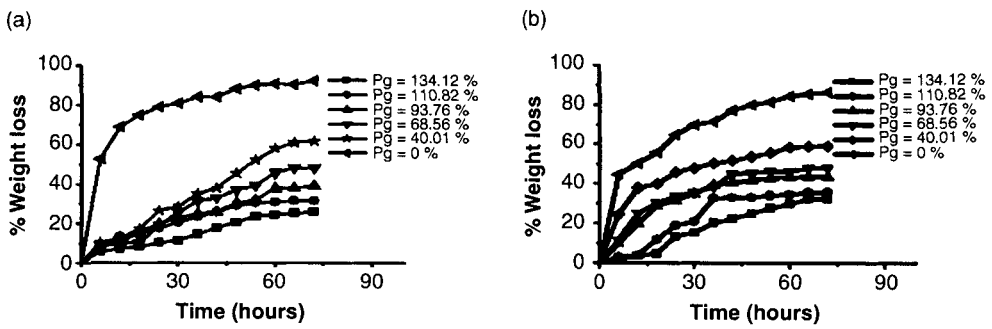


Figure 2.9 Chemical resistance studies of SPC graft copolymers (a) acid resistance studies (b) base resistance studies [55, 56].

of the graft copolymers. Thus, with increase in graft copolymerization there is increased alkali resistance [55, 56, 60, 78–80) (Figure 2.9).

2.5.2.3 *Electrical Stimulus Sensitivity*

Grafted and crosslinked polysaccharides are found to exhibit both AC and DC current based electrical stimulus sensitivity towards their deswelling behaviour. Moreover, deswelling is found to be dependent upon applied voltage and time of current flow. The deswelling behaviour of grafted polysaccharide could be due to the diffusion of H^+ and OH^- towards opposite electrodes which results in the easy escape of such ions out of the matrix. Consequently, there is an increased rate of deswelling [81–83] (Figure 2.10).

2.6 Applications of Modified Polysaccharides

Modified polysaccharides have diversified properties and are very significant from a technological point of view. These biopolymers can be used for various applications.

2.6.1 Sustained Drug Delivery

Functionalized biopolymers have successfully taken over the era of synthetic polymers because they are cost effective, biodegradable, environmentally friendly and efficient. These biopolymers, after graft copolymerization and crosslinking, can be used for the sustained release of drugs. Based on relative rates of diffusion (R_{diff}) and rate of polymer relaxation (R_{relax}), the diffusion of drugs depends upon the value of Diffusion Exponent (n). If $n < 0.5$, the $R_{diff} < R_{relax}$ and if $n \geq 1.0$, the $R_{diff} > R_{relax}$, whereas in case of $n > 0.5$ and < 1.0, $R_{diff} \approx R_{relax.}$ Thus, Fickian, Case-II and non-Fickian mechanisms are followed for the sustained release of different drugs in various media [41, 84, 85]. Moreover,

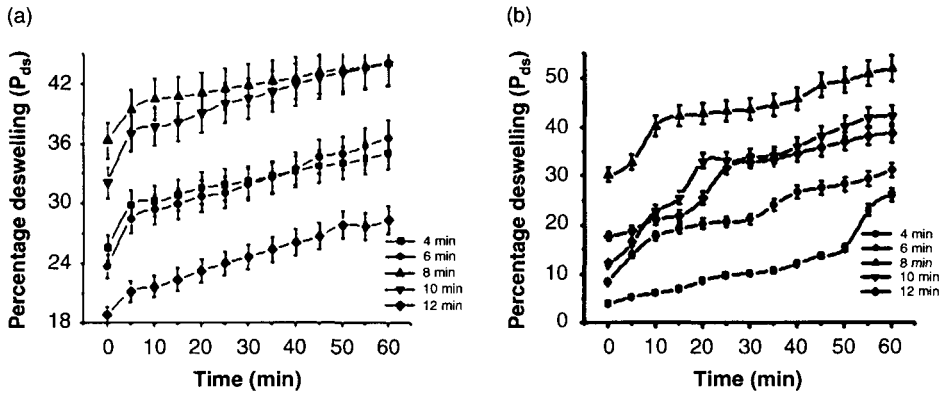


Figure 2.10 Electrical stimulus studies (a) under DC voltage (b) under AC voltage on Gum ghatti crosslinked graft copolymer [81-83].

the rate of diffusion of the drugs depends on the chemical and physical interactions between the polymer matrix and the drugs.

2.6.2 Controlled Release of Fungicide

Recent years have witnessed tremendous applications of pesticides, herbicides, fungicides and fertilizers. These chemicals are exploited without considering the disadvantages. After being used for about two or three decades, the world is witnessing the hazardous effects of these chemicals. Controlled release of fungicides through modified polysaccharides can thus be the major remedial factor which can occur through diffusion, degradation or a combination of both. Controlled fungicide delivery by the use of polymeric materials has become a field of much research interest [24, 41]. Release of fungicide through polymeric matrix depends upon the diffusion coefficient (D) which in turn depends upon the Diffusion Exponent (n) value. Thus, the Fickian, non-Fickian or Case-II diffusion can be ascertained from the values of 'n'.

2.6.3 Selective Removal of Water from Different Petroleum Fraction-water Emulsions

Three-dimensional crosslinked polymeric networks, also known as 'hydrogels', have the property of water absorption many times their own weight, and are known as 'superabsorbents'. These hydrogels are highly selective in nature and can remove saline from petroleum fraction-saline emulsions. Such modified polysaccharides are of great importance in the oil industries. Strong petroleum fraction-saline emulsions are a big headache for the oil industries and are difficult to break. The breaking of such strong emulsions requires a lot of energy consumption. However, crosslinked grafted polysaccharides can be effectively utilized without any consumption of energy for removal of water/saline from such oil-saline emulsions. Moreover, these polymers are biodegradable in nature; hence, they are a green technology [49, 50, 86–89].

2.6.4 Removal of Colloidal Particles from Water

Hydrogels have the characteristics to trap the colloidal particles in their folds and force them to settle down at the bottom. Thus, modified polysaccharides play an important role in the removal of colloidal particles from potable water. Since these polysaccharide devices are biodegradable and green in nature, they are effective substitutes for synthetic flocculants [84].

2.6.5 Graft Copolymers as Reinforcing Agents in Green Composites

Polymer composites reinforced with natural fibers have received considerable attention. Polysaccharides have attracted the attention of scientists and

technologists all over the world for energy extensive applications. Grafting of polysaccharides can further improve their properties such as water repellency, acid-base resistance, thermal stability and increased mechanical strength [90, 91]. Moreover, the grafted polysaccharides when used as reinforcing materials for the preparation of biodegradable composites are found to possess better compatibility with the matrix (Figures 2.11a–2.11f). Figures 2.11a–2.11c show the SEM of smooth area of pure polyhydroxybutyrate (PHB) matrix, sunn hemp (SH) fiber reinforced composites and grafted SH reinforced composites. Figures 2.11d–2.11f show the SEM of fracture area of pure PHB matrix, SH fiber reinforced composites and grafted SH reinforced composites. These micrographs clearly show the difference in their surface morphology. The pure matrix (Figures 2.11a and 2.11d) is free from any fiber and the surface is very smooth in comparison to SH fiber reinforced composites. SH fiber reinforced composites (Figures 2.11b and 2.11e) show the considerable amount of binding of fiber bundles and matrix. It has been found that graft copolymers showed better binding with matrix as their surface is amorphous and rough (Figures 2.11c and 2.11f) in comparison to original SH fiber. Figure 2.11f shows the strong binding of fiber and matrix in the fracture surface of graft copolymer reinforced PHB composites [54].

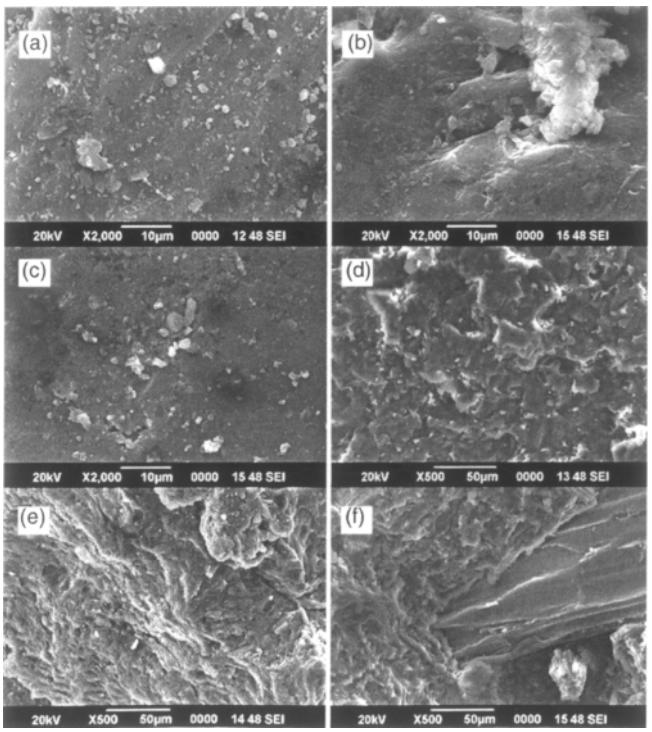


Figure 2.11 SEM of (a) PHB (b) SHF-PHB (c) SHF-g-copolymer-PHB smooth surface of composites and (d) PHB (e) SHF-PHB (f) SHF-g-copolymer-PHB fracture surface of composites [54].

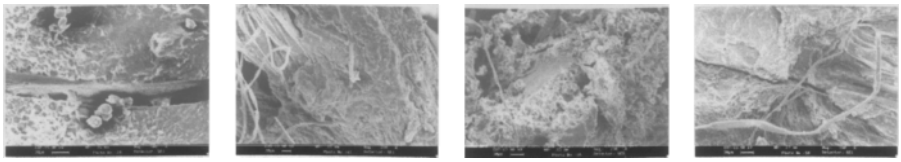


Figure 2.12 SEMs of different biodegradation stages of Corn starch composites [107].

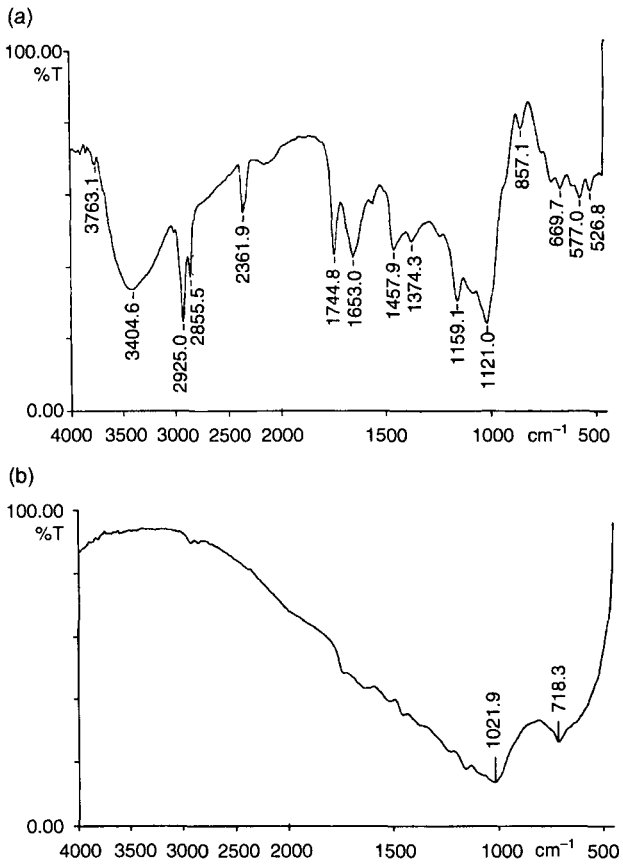


Figure 2.13 FTIR of different biodegradation stages (a) corn starch matrix composite (b) biodegraded corn starch matrix composite [107].

Graft copolymer reinforced composites are found to possess better mechanical properties such as compressive strength, tensile strength, elongation at break, abrasion resistance and hardness. Materials derived from natural sources and reinforced with different graft copolymers of polysaccharides are found to be green composites possessing good mechanical properties as well as being biodegradable in nature. These composites have good thermal stability along with water repellency and stability towards chemicals [92–108].

2.7 Biodegradation Studies

The over growing environmental pressure caused by the wide spread consumption of petroleum based polymers and plastics has hastened the development of biodegradable and environmentally acceptable materials. Biopolymers derived from various natural resources such as proteins, cellulosics, starch and other polysaccharides are regarded as the alternate materials. Biodegradable polymeric materials derived from renewable sources are the most promising materials because of their easy availability and cost effectiveness. Biodegradable modified polysaccharides have been found to possess varied applications such as salt resistant absorption of water [109].

Saccharum spontaneum graft copolymers reinforced with corn-starch matrix-based green composites studied for biodegradation through composting method, have shown promising results. Analysis of different biodegradation stages was carried out using FT-IR and SEM techniques [107]. Soy protein concentrate graft copolymers and their composites are found to be biodegradable in nature in both composting and soil-burial methods (Figures 2.12 and 2.13). Similar results can be seen in the graft copolymers of *Gum ghatti.*

2.8 Conclusion

Polysaccharide graft copolymers have a large number of advantages over natural backbones. These polymeric materials are stable against acid-alkali attack; they are moisture retardant and have thermal stability. Other applications of crosslinked graft copolymers include sustained drug delivery, controlled fungicide release, selective absorption of water from petroleum-water emulsions and purification of potable water. Moreover, such graft copolymers can be used as reinforcing materials in the preparation of green composites. These composites are biodegradable and have chemical resistance and promising mechanical properties.

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3

Natural Polysaccharides: From Membranes to Active Food Packaging

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Abstract

Biopolymers are gaining popularity in various applications due to their renewable nature, biocompatibility and biodegradability. Polysaccharides are a major class of biopolymers with diverse biological functions primarily due to their chemical structure and reactivity with other compounds. An increasing amount of research is focused on understanding these natural functions, and fabricating new materials exhibiting these enhanced properties in the fields of ionically selective membranes and active food packaging.

Modified polysaccharide biopolymers have found applications from permselective membranes to ionically conductive membranes for fuel cells. In this review, ionically selective membranes in particular will be explored. Recent studies and advances in using modified polysaccharides for food applications will also be reviewed with specific attention given to their potential uses in the development of active food packaging solutions, which include antimicrobial systems, coatings and bioactive compound delivery systems.

Keywords: Biopolymer, chitosan, active food packaging, membrane, permselective, antimicrobial, bioactive

3.1 Introduction

Biopolymers are renewable, biodegradable and biocompatible molecules and have been the focus of numerous research studies in the past decade. The presence of reactive functional groups increases their popularity for a wider range of applications in the biomedical, chemical engineering and food science fields. Two common and abundant biopolymers are the polysaccharides: cellulose and chitosan. To better understand their popularity, it is important to know their sources and chemistries.

Cellulose, (Figure 3.1), is the most abundant natural polymer and consists of β-(1→4)-*D*-glucose linkages that are majorly found in plant cell walls. Properties of cellulose depend mainly on its source and degree of polymerization. Chitin (*N*-acetylglucosamine), (Figure 3.2), the second most abundant

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (59–80)

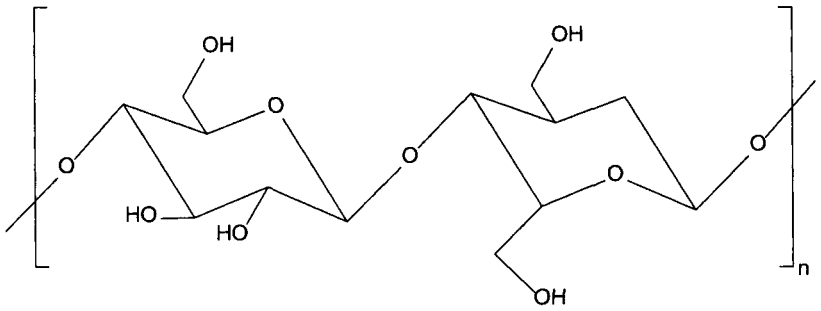

Figure 3.1 Chemical structure of cellulose.

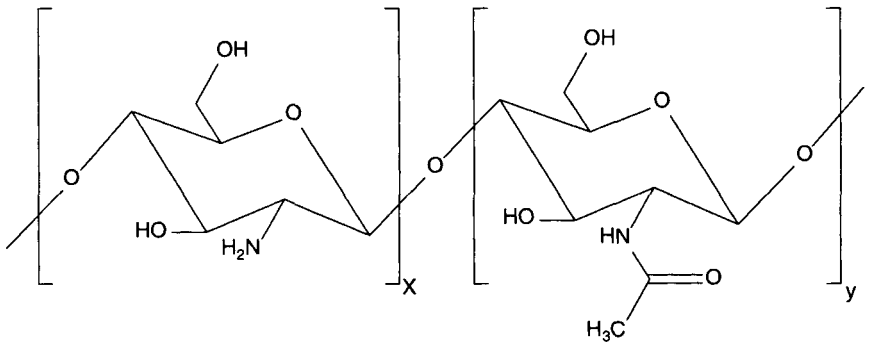

Figure 3.2 Chemical structure of chitin (X<Y) and chitosan (X>Y).

biopolymer, is found in exoskeletons of crustaceans and cell walls of fungi. However, chitosan, the deacetylated form of chitin, is favored due to its increased solubility in acidic solvents. Chitosan consists of β-(1→4) linkages of *N*-acetyl-*D*-glucosamine and *D*-glucosamine, (Figure 3.2). Its amine functional group enhances the biopolymer's reactivity. The molecular weight (MW) and percent deacetylation (%DD) of chitosan influence its properties and behavior in solution. The broad range of interactions that cellulose and chitosan can form with other chemical species drives the increasing interests of researchers to explore its potential uses in various fields.

The following sections of this chapter will look at recent developments of both cellulose and chitosan for specific application in water purification, energy and food storage as: (1) permselective membranes for chemical sensing and polymer electrolyte membranes for fuel cells and; (2) antimicrobial/lipid barrier films for active food packaging.

3.2 Polysaccharide Membranes

Fresh water scarcity and the alleviation of petrochemical dependence are currently two of the most pressing engineering challenges. According to *The World's Water 2008-2009 Report*, "by 2025, 1.8 billion people will be living in

regions with absolute water scarcity and two out of three people in the world could be living under conditions of water stress [1]." The US Department of Energy has set optimistic goals for hydrogen fuel cell vehicles with greater than a 300 mile range and 5,000 hour fuel cell durability [2]. Two large areas of development for polysaccharide polymers are permselective membranes for water purification and filtration and polymer electrolyte membranes (PEMs) for fuel cell applications.

3.2.1 Permselective Membranes

A permselective membrane is an ion-exchange material that allows particles of a particular charge state to selectively pass through or attach onto it [3]. A simplified view of a cationic permselective membrane is shown in Figure 3.3, and displays the selective rejection of negatively charged particles from a membrane, allowing unperturbed attachment of positively charged particles to active sites. This concept has been used to form charge based electrochemical sensors [4] for compounds such as testosterone [5], carcinoma antigens [6] and human immunodeficiency virus (HIV) [7]. Each of these sensors utilizes either a cationic or anionic specific membrane that will allow the desired analyte to penetrate to an immobilized functional group or enzyme, which binds the analyte. The substrate is normally conductive, which allows electrochemical analytical techniques to be utilized to determine analyte concentration levels.

3.2.2 Ionically Conductive Membranes

To understand the modifications made to polysaccharides in PEMs applications, a cursory knowledge of fuel cells is necessary. A fuel cell is an electrochemical cell that converts chemical fuel into electrical energy. Figure 3.4 shows a simplified view of a proton conductive fuel cell. The main components in a PEM fuel cell are catalyst layers, gas diffusion layers and the PEM itself. These three components comprise the membrane electrode assembly. The catalyst

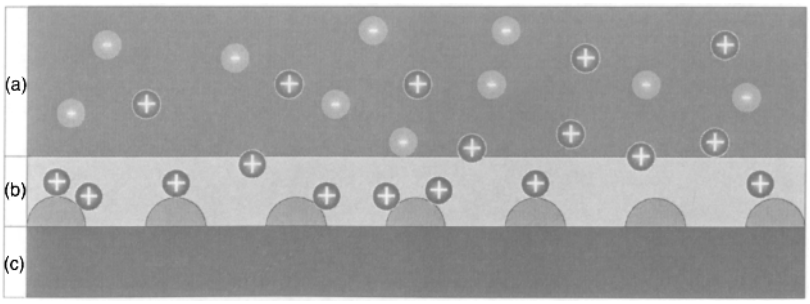

Figure 3.3 Permselective membrane: (a) solution containing positive charged analyte and negatively charged secondary components; (b) cationic permselective membrane, half-circles indicate active sites; (c) conductive substrate.

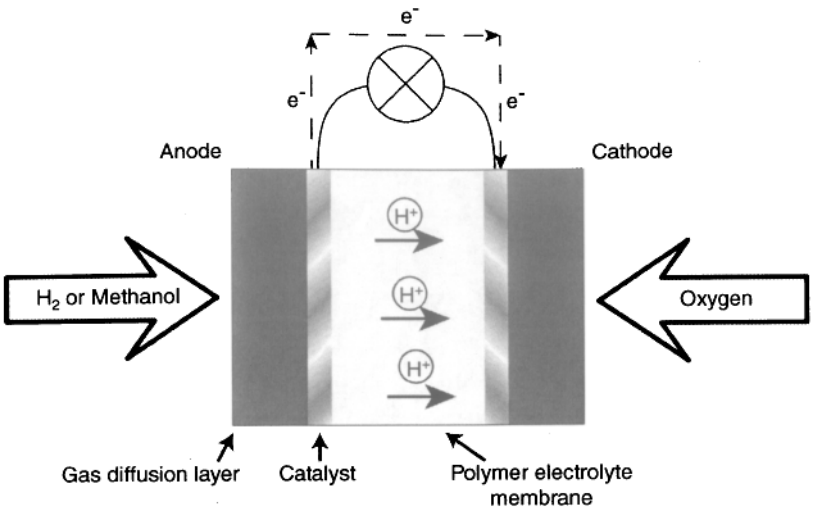

Figure 3.4 Schematic of polymer electrolyte membrane fuel cell.

layers act as electrodes and typically consist of platinum or platinum alloys. Gas diffusion layers are carbon fiber cloths that act as a medium to disperse the fuels evenly across the polymer electrolyte membrane. The polymer electrolyte membrane acts as a proton conductor and barrier to unwanted molecular transport. The proton conductive PEM typically consists of strongly acidic groups, which aid in proton transport through the membrane. The fuel cell produces power *via* an electrochemical reaction in which a fuel is oxidized at the anode to produce protons and electrons. Protons travel through the PEM where they react with oxygen at the cathode to produce water. Electrons travel along an external circuit to power the load attached to the fuel cell.

Ionically conductive membranes fall into two categories: cation conductors and anion conductors. In a PEM fuel cell, which contains a cationically conductive membrane, the exchange media is a proton (H^+), typically provided from a fuel source of hydrogen. The hydrogen is catalytically split into protons and electrons at the anode. The protons permeate the exchange membrane and the electrons are provided with an external circuit terminated at the cathode, where the protons recombine with the electrons and an oxygen atom to form water (Equations 3.1, 3.2).

$$\text{Anode} \qquad H_2 \rightarrow 2H^+ + 2e^- \tag{3.1}$$

$$\text{Cathode} \qquad \tfrac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O \tag{3.2}$$

In an alkaline fuel cell, which contains an anionically conductive membrane, the exchange media is a hydroxyl radical, (^-OH). At the anode, hydrogen is oxidized through a redox reaction, (Equation 3.3), producing water and

releasing two electrons. The electrons are provided an external circuit to follow, terminating at the cathode with an oxygen reduction reaction (Equation 3.4).

$$\text{Anode} \qquad H_2 + 2OH^- \rightarrow 2H_2O + 2e^- \tag{3.3}$$

$$\text{Cathode} \qquad O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \tag{3.4}$$

Charge alone is not sufficient to induce ionic conductivity. Ion exchange capacity (IEC), water uptake, and water retention capabilities help to ensure good electrochemical properties such as membrane conductivity. As water uptake and water retention properties increase in the bulk membrane, the conductivity tends to increase proportionally. IEC provides information regarding the density of ionizable hydrophilic groups in the membrane matrix, which are responsible for the conduction of protons and thus IEC is an indirect and reliable approximation of the proton conductivity [8].

3.2.3 Polysaccharide Polymers

Due to their renewable nature and ready functionality [9], polysaccharide based systems have gained increasing attention for use in permselective membranes and polymer electrolyte membranes (PEMs). Polysaccharides with high levels of amino and hydroxyl functional groups such as chitosan and cellulose are easily functionalized [4, 10]. High levels of functionality are paired with strong hydrophilicity, good film-forming character, and excellent chemical resistance properties to make polysaccharides very attractive in membrane research [10d].

Much attention is paid to using polysaccharide membranes to selectively reject, entrap, or otherwise interact with ions depending on charge or element [4,10d, 10f, 11]. For a permselective membrane used in an electrochemical sensor, analyte possessing a certain charge state is allowed to pass through the membrane and is detected by voltammetry or similar electrochemical techniques. In the case of PEMs, it is desirable to have a membrane, which will allow either an anionic or cationic species to pass through while rejecting its opposite. In PEMs, much attention is paid to the rate of ionic transport. The commonality in these methods is that the polysaccharide membrane is modified in physical conformation, as well as chemical structure, to achieve selectivity.

3.3 Permselective Membranes

Several methods have been shown to induce charge-based selectivity in a polysaccharide membrane. Methods explored by Saxena *et al.* were to functionalize chitosan with either phosphonic acid groups or quaternary ammonium groups and crosslink with poly(vinyl alcohol) as shown in Figure 3.5 [10a].

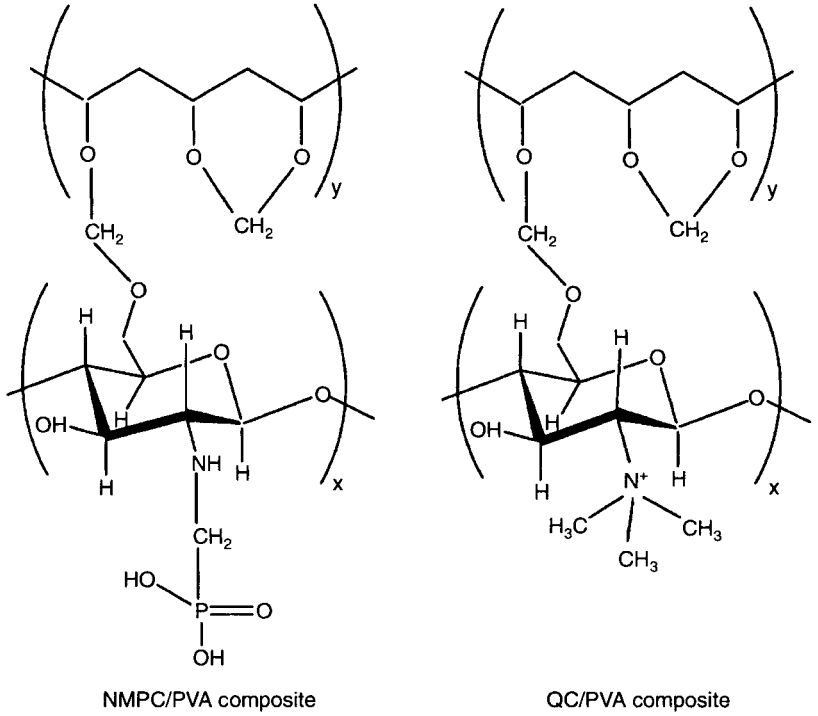

Figure 3.5 Schematic structure of NMPC/PVA and QC/PVA composites [10a].

Saxena *et al.* produced a permselective membrane from a combination of these oppositely functionalized chitosan chains. Both modifications were found to have good hydrophilic interaction with water and the mixed solution resulted in charge neutralization. The resultant membrane was shown to discriminate between different ionic radii, specifically perturbing the diffusive properties of molecules with a larger ionic radius such as Mg^{2+} and Ca^{2+}, while facilitating the diffusion of Na^{+}, which has a smaller ionic radius. This implies the suitability of the bimodal functionalized chitosan membrane for separation of Na^{+} from Ca^{2+} and Mg^{2+}, allowing for specific detection of Na^{+}.

In order to create a permselective membrane by means of physical conformation, Thielemans *et al.* produced cellulose nanowhiskers derived from cotton and formed them into nanostructured thin films for use in cyclic voltammetric tests [4]. To produce nanowhiskers, cotton wool was hydrolyzed using sulfuric acid and the resultant nanowhiskers were neutralized, purified, and ion-exchanged to replace surface cations with hydrogen_ENREF_2. The nanowhiskers were then suspended and drip coated onto a glassy carbon substrate. These thin films of cellulose nanowhiskers were stabilized by extensive hydrogen bonding, causing full water insolubility. In voltammetric testing, the nanowhiskers were found to cause the formal potentials of the anionic and cationic mediators to shift to more positive and more negative potentials, respectively, while not affecting the neutral species. Expanding these studies, these dispersions of cellulose nanowhiskers coupled with a glassy carbon electrode

could lead to sensor devices that selectively accumulate certain species while simultaneously excluding other interfering species.

3.4 Ionically Conductive Membranes

While permselective membranes used in sensor applications focus mainly on the exclusion of certain charge states and inclusion of other charge states, research into ionically conductive membranes focuses primarily on enhancing the transport of charged ions across a membrane.

3.4.1 Cation Conductive Membranes

While Nafion®, a perfluorinated polymer developed by DuPont, is the most commonly used proton conductive polymer electrolyte membrane; it is an insufficient solution in a number of areas. It has high cationic transport (approximately 9.56 S/cm) [8] but also has high levels of methanol fuel crossover, slow anode kinetics and very high cost [12]. Fuel cell membrane performance can be estimated from the ratio of proton conductivity (σ) to methanol permeability (P). The higher the value of σ/P, the better the membrane performance would be [13]. Chitosan has been shown to have a much lower methanol permeability than Nafion®[14], and as such, a great deal of attention focused on developing chitosan membranes with high levels of ionic conduction and low methanol permeability as delineated in Table 3.1.

The work of Binsu *et al.* has focused on the phosphonic functionalization of chitosan combined with polyvinyl alcohol and has yielded the highest fuel cell

Table 3.1 Modifications to chitosan to induce cationic conductivity.

Modification	Conductivity Type	Measured Conductivity (S/cm)	Methanol Permeability (cm^2/s)	Fuel Cell Membrane Efficiency
Base Nafion®-117	Proton [8]	9.56×10^{-2} at 25°C	13.10	7.30×10^{-3}
Phosphonic chitosan/PVA complex	Proton [8]	Up to 4.29×10^{-2} at 25°C	1.03	4.17×10^{-2}
Phosphotungstic acid	Proton [21]	2.4×10^{-2} at 25°C	3.3	7.27×10^{-3}
Heteropolyacid	Proton [23]	1.5×10^{-2} at 25°C	3.8	3.95×10^{-3}
Chitosan-oxide composite	Proton [24]	10^{-3} to 10^{-2} at 25°C	Not reported	Not reported

membrane efficiency of all reported chitosan based cationic membranes. This was seen to be a result of the relatively high IEC, and high water uptake and retention properties [8]. Work by Cui *et al.* has delved into the incorporation of phosphotungstic acid into a chitosan membrane [14a]. This membrane was a polyelectrolyte complex between phosphotungstic acid and the amine groups of chitosan and formed a stable membrane with persistent negative charge. Many other techniques have been attempted to induce cationic conductivity such as including heteropolyacids, and combining metal oxides with a chitosan composite membrane, and all have been successful to varying degrees, but all have centered on the addition of localized negative charge centers. To induce protonic conductivity into a membrane, persistently negatively charged groups are added to the polymer. This is exemplified in Table 3.1 as each of the proton conductive groups added was negatively charged, and would remain charged in a wide pH range.

3.4.2 Anion Conductive Membrane

Nearly all of the attention on anion exchange PEMs has been on adding quaternary ammonium groups, which retain a positive charge in most environments. A technique developed by Lim *et al.* [15] to add quaternary ammonium groups to chitosan uses glicydyltrimethylammonium chloride and adds the functional group to the amine on chitosan as shown in Figure 3.6. The anionic conduction associated with this group, in conjunction with the lower methanol permeability of chitosan, has been shown to create membranes with anionic conduction on the same order as the industry standard Nafion® [15-16] (Table 3.2).

Overall, a great deal of attention has been paid to inducing ionic conductivity in chitosan membranes for application in fuel cell membranes. In both cationic and anionic membranes, the fuel cell performance values are approaching that of the industry standard Nafion® membranes.

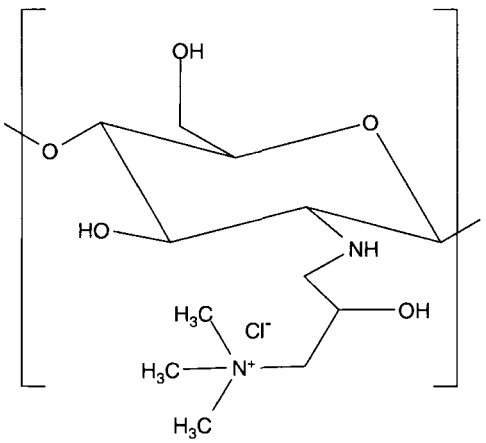

Figure 3.6 Quaternized chitosan.

Table 3.2 Anionic conductivity values for quaternized chitosan.

Modification	Conductivity Type	Measured Conductivity (S/cm)
Base Nafion®-117	Anion [27]	8.0×10^{-2}
Quaternary ammonium	Anion [28]	3.0×10^{-2}
Quaternary ammonium	Anion [27]	0.9×10^{-2}
Quaternary ammonium	Anion [26]	0.71×10^{-2}

3.5 Polysaccharide Membranes: Synopsis

Polysaccharide polymers have shown great promise as ionically selective membranes. While permselective polymer membranes have been in literature for over 50 years, the last 10 years has seen a great deal of development with polysaccharide polymers specifically. In permselective membranes, they have shown immense potential as charge sensitive semi-permeable membranes for sensing applications. In ionically conductive polymer electrolyte membranes, modified chitosan has shown nearly equivalent performance characteristics to the industry standard Nafion® at a significantly lower cost. While carbohydrate based PEMs have the benefit of lower cost, membrane efficiencies will need to reach and exceed those of Nafion® to reach the goals set forth by the Department of Energy. Protonic and anionic conductivity will also need to improve, because without improving this, power levels delivered from the PEM fuel cells may not be sufficient for certain applications.

There have been major developments in polysaccharide membranes in recent years, but there are still a number of shortcomings that will need to be overcome. The engineering challenges are significant, but given the rapid development over the past ten years and the increase in need for new power conversion sources and fresh water, these challenges should be surmountable.

3.6 Active Food Packaging

Natural polymers are not newcomers in food packaging. Commercial film and paper-based packaging using cellulose or starch is a big industry worldwide. The *2002 Industry Status Report* of the US Flexible Packaging Association has reported that in the US alone, the flexible packaging industry, which includes food packaging materials, is worth around $20 billion US dollars.

Economic issues are just one side of the whole food packaging concern. The move to more environmentally friendly food manufacturing practices has slowly changed the way that food is being packaged. Consumers generally prefer fresh food products and prefer recyclable or biodegradable packaging. The same consumers however also consider food safety. This has been

a balancing act to both consumers and manufacturers and has driven food packaging research to newer areas – active and biofood packaging.

Active food packaging is a film or coating that is directly or indirectly in contact with any food surfaces to increase the shelf life or improve the quality of the packaged food. The big classifications fall under either oxygen-scavenging or antimicrobial but other upcoming types such as ethylene-scavenging, moisture absorbers, taint removers, and color and flavor enhancers have also been identified.

In the case of natural polymers, polysaccharides like cellulose and chitosan are widely studied as antimicrobial films and barrier/coating materials. The following section reviews the recent studies on cellulose and chitosan having current and potential use as antimicrobial material for active food packaging systems.

3.7 Antimicrobial Films

Much of the recent studies on active packaging involve the development of antimicrobial films or coatings. The reason is twofold: first, it lessens the need and cost to add preservatives to the packaged food, and second, natural and safe polymers have shown promising antimicrobial properties that may inhibit the growth of both spoilage and pathogenic microorganisms that may deteriorate the food and cause food borne illnesses, respectively.

Common food-borne microorganisms. Before delving into a short review of recent developments in antimicrobial packaging, it is important to present a short background about microorganisms found in food. Antimicrobial products added to food commodities generally target two types of microorganisms – pathogenic or spoilage organisms. Often, bacteria can be of both types. Food safety is often concerned with making sure that food is safe from manufacture until consumption. Tests to detect specific pathogens and spoilage microorganisms for various food systems are standard and required by the US Department of Agriculture (USDA) or the US Food and Drug Administration (FDA).

The most common tests involve examinations of yeast, mold, *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus* and *Listeria* spp. Depending on the food product under study, the tests are often for detection or enumeration of microbes. Yeast and molds are a public health concern due to their ability to produce mycotoxins, which are not destroyed by standard food processing methods. Mycotoxins are poisonous and can cause acute or chronic health effects. *E. coli*, which includes the O157:H7 strain, is a known pathogen that is strictly regulated by the USDA. *E. coli* are described as Gram-negative, non-spore forming, facultative anaerobes, or aerobic, rod-shaped, and are able to utilize lactose and produce gas or acid. Moreover, *E. coli* is used as an indicator organism to test for coliform. Naturally found in the gut, a positive result for *E. coli*/coliforms indicates contamination from fecal sources (e.g. water source used manufacturing or food handlers not washing hands when preparing food).

Like *E. coli*, *Salmonella* spp. is a member of the *Enterobacteriaceae* family often isolated from humans and food products. The various types of *Salmonella* spp are known pathogens. *S. typhi* causes typhoid fever while other species cause diarrhea or even septicemia. Its detection and identification therefore is important for food safety. Common food products that harbor *Salmonella* spp include eggs or poultry products that have not been properly processed or cooked.

Other bacteria, *S. aureus*, can also get into food by unsanitary processing conditions. Though these bacteria do not grow in refrigerated conditions, they can grow in processed or cooked food and even those commodities with low moisture-content (i.e. high salt) foods, separating them from other known pathogens. Some species or strains of S. aureus also produce enterotoxins, which when present in food, can cause vomiting or diarrhea if consumed. It should be noted though that this type of intoxication is caused by a large amounts of the toxin (10^6 CFU/g), which necessitates not just detection but the enumeration of the organisms to determine if the food product underwent thermal abuse.

Listeria spp. is probably one of the most seriously treated food-borne pathogens due to its ability to cause listeriosis, a condition with flu-like symptoms which can compromise the human immune system especially in pregnant women, children and the elderly. Mortality rates from full listeriosis are at 20% prompting the USDA and FDA to set a "zero tolerance" against the pathogen. Detection of this microbe is therefore more important than enumeration. In food, *Listeria* spp. can grow even at refrigerated temperatures and have been isolated from meat and cheese products.

Most strains or species of these microorganisms also cause spoilage by utilizing and degrading the important food components for their growth and producing acids or gas that changes the pH of entire the food, leading to changes in texture, taste and properties of the components. Considering all the potential harm brought about by pathogenic or spoilage microbes, it is not surprising that the food packaging industry is trying hard to help address food safety concerns as well as keeping materials safe for the environment.

The ongoing efforts have been promising, especially in the antimicrobial food packaging area. For this part, we would like to summarize the recent developments on antimicrobial systems utilizing chitosan, cellulose, their derivatives and blends (Table 3.3).

3.7.1 Chitosan

Chitosan owes its broad-spectrum antimicrobial property to the amino groups present on the chain[17] (Figure 3.7). This implies that the %DD and MW affects this property but only a few studies [18] have tried to explain its relationship. There are two main mechanisms that have been suggested to explain the action of chitosan against microbial cells: a) electrostatic membrane binding; and b) cell nucleus permeation. The first mechanism is due to the interaction of chitosan's cationic amino groups with the anionic groups on the surface of microbial cells. This interaction creates a layer around the cell wall that inhibits

Table 3.3 Recent developments in chitosan (CS), cellulose and their derivatives and blends as antimicrobial films/coatings.

Polysaccharide	Additives or Blends	Microorganisms Tested	Ref.
Chitosan and derivatives			
CS	(in dongchimi)	*Leuconostoc mesenteroides* *Lactobacillus plantarum*	30
CS	thyme oil clove oil cinnamon oil	*Listeria monocytogenes* PTCC 1298 *Staphylococcus aureus* PTCC 1431 *Salmonella enteritidis* PTCC 1318 *Pseudomonas aeroginosa* PTCC 1344	31
CS	palmitic acid O,O'-dipalmitoylchitosan	*Salmonella typhimurium* *L. monocytogenes*	32
CS	silver nanoparticles	*Escherichia coli*	33
CS	silver	*S. aureus* ATCC 25923 *S. aureus* ATCC 6538P	34
CS	jute fabrics (cellulose) Ag^{+1}, Zn^{+2}, Zr^{+2}	*Staphylococcus aureus* *Candida albicans*	35
CS	polyvinyl alcohol glutaraldehyde	*E. coli* *S. aureus* *Bacillus subtilis*	36
CS	sorbitol	Aspergillus niger	37
CS	starch Ag^{+1} nanoparticles	*E. coli* *S. aureus* *Bacillus cereus*	38
CS acetate	None	coated on sweet cherries (*Prunus avium* L.)	39
CS acetate	None	*Listeria monocytogenes CECT 86* *Salmonella spp CECT 554* *S. aureus CECT 5672*	40
Cellulose	nisin	*L. monocytogenes*	41
Cellulose	pediocin (ALTA 2351)	*L. innocua* and *Salmonella* sp.	42
Hydroxylpropyl methyl-cellulose (HPMC)	nisin glycerol	*Listeria* sp. *Staphylococcus* sp. *Enterococcus* sp. *Bacillus* sp.	43

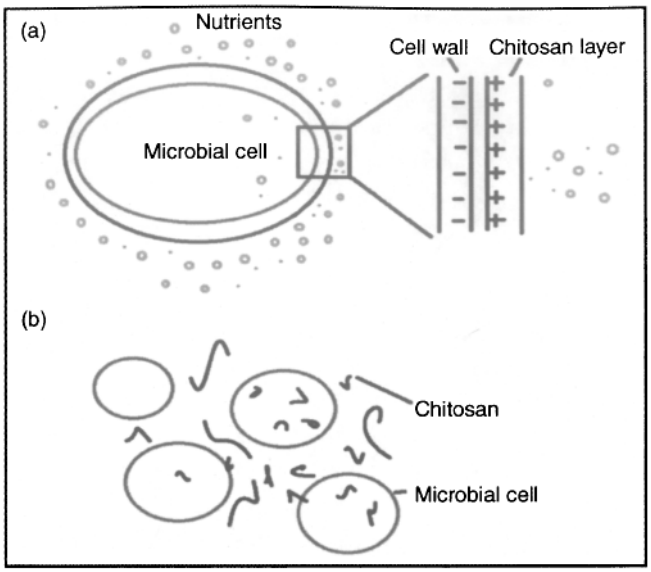

Figure 3.7 Suggested antimicrobial mechanism of the action of chitosan against microbial cells: (a) electrostatic binding to membrane; (b) permeation to cells.

essential nutrient transfer between the cell and its environment [19]. In this mechanism, the %DD majorly affects the property. The higher the %DD, the higher the electrostatic binding to the membrane leading to increased antimicrobial effect. The increased positive charge of chitosan also allows it to attach better to cell membranes [18b]. It must be noted though, that this property may be affected by the %DD and pH since it is dependent on the amine group. Also, the type of substitution may affect the cell adhesion. Chitosan can be substituted on the amine (*N*-substitution), hydroxyl (*O*-substitution) or both groups (*N,O*-substitution).

On the other hand, a few reports indicated an unclear relationship between %DD and antimicrobial activity. Hetero-chitosans, with different %DDs and MWs, revealed varied interactions to three Gram-negative and five Gram-positive bacteria. The authors found that chitosan having 75%DD is more effective in inhibiting bacterial growth than those having 50% or 90% DD. This suggests that activity may depend on the type of chitosan and microorganism used [18f].

The second mechanism is due to the permeation of low MW chitosan oligomers into the bacterial cell. This inhibits the synthesis of both proteins and RNA, which are essential processes in the cell. In this case, the MW majorly influences chitosan's antimicrobial property. High MW chitosans are expected to have low permeation into the cell nucleus leading to low antimicrobial activity. This has been exhibited by labeled chitosan oligomers (5-8kDa) found inside *E. coli* cells [18a]. Aside from these two mechanisms, several authors have suggested that the chelating property of chitosan possibly slows down the growth of microorganisms [20]. However, this mechanism still remains unclear.

A recent study conducted by Lee *et al.* [21] utilized the antimicrobial property of chitosan to control lactic acid bacterial growth in dongchimi, a popular type of the Korean kimchi. Dongchimi is usually processed by fermentation, but excessive bacterial growth is often blamed for the food product's undesirable taste, odor and texture. Chitosan prepared from chitin was directly added before fermentation to act as a natural preservative. MW and chitosan concentrations were the main factors affecting its activity. Optimum concentrations that inhibit bacterial growth were noted at 40mg/L at 140°C and 10min fermentation conditions but higher concentrations of up to 1000mg/L were needed in order to achieve extended improvements in taste after fermentation.

Currently, researchers are trying to increase the low antimicrobial activity of chitosan to compare with commercially used chemicals. The recent advances include the use of composites and other biologically active substances (Table 3.3) that are known antimicrobial agents. The expected outcome is an increased antimicrobial effect due to the synergistic action of both antimicrobial agents. Moreover, the use of lesser synthetic agents to improve the naturally-derived polymer can be both economical and attractive.

Essential oils and other lipids. Essential oils like thyme, clove and cinnamon, at low concentrations (0.5-1.5%v/v) have been added to chitosan films to improve antimicrobial and barrier properties [22]. These extracts contain phenolic groups that may be fundamental to their antimicrobial action. Generally, these oils are effective against Gram-positives also observed in the films containing chitosan. In the study, thyme essential oil had the greatest antimicrobial effect against both Gram-positive and Gram-negative bacteria. It is also interesting to note that though the antimicrobial effect was increased for CS films with thyme and clove (both hydrophilic), the moisture content, solubility of film in water and water vapor transmission rates increased, leading to poor barrier properties. CS-cinnamon films however have the opposite effect, having poor antimicrobial activity but improved water barrier property compared to thyme and clove oils. This was due to oxalic acid present in cinnamon oil that could have crosslinked chitosan and prevented antimicrobial action [22] Aside from improving antimicrobial properties, lipids are added to chitosan to improve the barrier properties. Quite a few strategies have been demonstrated, which are summarized in Table 3.4.

For food packaging applications, increasing the hydrophobicity of chitosan for improved barrier properties has been a challenge. Acetylation or acylation reactions at the amine groups increase the solubility of chitosan in organic solvents and improve its nonpolar interactions. This enables chitosan to react better with lipids and enhance its barrier properties. However, if both antimicrobial properties are also desired, the amine groups have to be free. Bordenave *et al.* [23] demonstrated how both of these properties can be simultaneously enhanced by the *O'O*-acylation of chitosan in a biphasic media. They compared how direct incorporation of palmitic acid and chemical modification by grafting of *O,O'*-dipalmitoylchitosan (DPCT) to chitosan affects the vapor, water and oil barrier characteristics and the antimicrobial efficacy of the films against *S. typhimurium* and *L. monocytogenes*. The vapor barrier properties did

Table 3.4 Chemical modification of chitosan for improved reactivity.

Modifications	Modified Property	Ref.
Hydrolysis	Solubility Biological activity	53–55
O-hydroxyalkylation	Solubility	53,56,57
Reductive *N*-alkylation	Metal chelation	53,58,59
O- and *N*-carboxyalkylations	Anionic property	53,60–62
Tosylation	Protective method for further reactions	53,63
N-phthaloylation	Protective method for further reactions	53,64–67
Acetylation	Hydrophilicity/hydrophobicity	68,69
Acylation	Hydrophilicity/hydrophobicity	32,70

not change with chitosan-palmitic acid but deteriorated with chitosan-DPCT. In the case of water, oil-barrier and antimicrobial properties, both types of lipids enhanced all these. Interestingly, the films were able to inhibit >98% of the culture bacteria even though the increased hydrophobicity can be a challenge in making sure that contact occurs between the film and cultures on agar.

Silver nanoparticles. Ag^{+1} nanoparticles are well known antimicrobial agents, with the highly reactive silver (I) ion (Ag^{+1}) interacting with the functional groups found on the microbial cell walls. This hinders nutrient transport to and from the cell leading to cell death [24]. Aside from its antimicrobial property, Ag^{+1} is attractive as a food preservation additive as it is non-toxic and non-inflammatory [25].

Recent studies have utilized chitosan's chelating property to develop chitosan-Ag^{+1} composite films with improved antimicrobial activities. Tankhiwale *et al.* [26] observed an almost log scale reduction through time of *E. coli* when Ag^{+1} nanoparticles were added to chitosan (from chitin, MW=400kDa) films. However, it is not clear if the inhibition was due to chitosan-Ag^{+1} or Ag^{+1} alone since the chitosan-only films did not indicate any significant bacterial growth reduction. To further investigate how bacterial cells respond, Diaz-Visurraga *et al.* [18g] used microscopy to investigate how the morphology of *S. aureus* cell walls change in the presence of chitosan (MW=170kDa and 400kDa, 95%DD) and Ag^{+1}. Cell growth varied depending on the strain, presence of Ag^{+1} and chitosan MW. Changes in cell morphologies were observed most on high MW-Ag^{+1} films than medium MW-Ag^{+1} ones. High MW chitosan-Ag^{+1} bind to the penicillin-binding protein 3 (PBP 3) of *S. aureus* ATCC 25923 causing lysis, cluster formation and cessation of septation, while the medium MW chitosan-Ag^{+1} binds to PBP 2 resulting in filament formation and deformation of *S. aureus* ATCC 25923 cells. The antimicrobial action of

the medium MW chitosan-Ag^{+1} films on ATCC 6538P strains is mainly due to disruption of nutrient transport in and out of the cells. The transmission electron microscopy (TEM) technique however is tricky, as the sample preparation has to be handled very carefully to reduce the introduction of artifacts from staining, cutting or mounting.

Though the use of Ag^{+1} is widespread in a number of areas including food packaging, the presence of chemical residues such as $NaBH_4$ [27], dimethylformamide [28] or formamide [28a] that are used to reduce silver nitrate is still a health and processing concern. Not only are these reagents toxic, their use also requires an additional removal process that is not cost and time effective. Newer technologies involving the reduction of silver nitrate by radiation revealed promising results. Films containing chitosan-starch and Ag^{+1} with improved antimicrobial activity against *E. coli*, *S. aureus* and *B. cereus* have been developed with a γ-irradiation process used to reduce silver nitrate in chitosan (95%DD, MW=700kDa) [29]. Yoksan *et al.* [29] further determined that the minimum inhibitory concentrations (MIC) of silver in the chitosan-starch films against the test bacteria was 5.64 μg/mL. The MIC is a common measurement to test how strong an antimicrobial agent is after 24h of incubation. Hence, antimicrobials with smaller MIC values are ones having the strongest antimicrobial activity. The 5.64 μg/mL is relatively strong compared to γ-irradiated chitosan-only solutions having MIC values of 312.50 μg/mL for *E. coli* or *S. aureus* and 625.00 μg/mL for *B. cereus* [29].

Metal ions. Chitosan is known to complex metal ions. The chelating ability of chitosan is superior compared to other known biopolymers [30]. This property is widely investigated for its use as sensors or filtration materials in heavy metal ion detection or separation [17, 20a, 31]. The binding of chitosan with metal ions is thought to provide donor atoms that may improve the antimicrobial activity of the material; however, studies on this area are quite limited [32].

Recently, cellulose-based fabrics treated with chitosan (82.9%DD, MW=160kDa) and different metal ions (Zn^{+2}, Zr^{+2} and Ag^{+2}) have been studied for use in antimicrobial textiles against *S. aureus* and *C. albicans*.

Other polymers. Other biocompatible polymers have also been added to chitosan films or fibers. Though these polymers do not have any additional antimicrobial activity, their addition to chitosan is expected to improve physicochemical and mechanical properties of the biopolymer. Tripathi *et al.* [33] used solutions and films of chitosan (79%DD) with polyvinyl alcohol (PVA) and the crosslinker glutaraldehyde against *E. coli*, *S. aureus* and *B. subtilis* in whole or sliced tomatoes. Interestingly, inhibition clearing zones were not observed around chitosan-PVA films in all three bacterial cultures while chitosan-PVA solutions have clear inhibition zones. Though the authors explained the reduced film activity due to the inability of chitosan to migrate across the agar and the need for chitosan-bacterial contact, we think that this might also be due to the reduction of available amino groups due to glutaraldehyde crosslinking. Though this is most likely possible, it also raises the argument that the toxic and unreacted glutaraldehyde is present in excess.

The electrospinning process has also been employed to fabricate mats having antimicrobial properties. Torres-Giner *et al.* [34] electrospun blends of zein and chitosan. The 25wt% zein:chitosan in ethanol:TFA (2:1 w/w) yielded fibers in the range of 128.5-92.3wt% depending on chitosan concentration. An increase in the amount of chitosan resulted in a decrease in the fiber diameter. The fibers were ribbon-like but beading was also observed. The addition of chitosan slowly increased the thermal resistance of zein fibers and showed antimicrobial activity against *S. aureus* at pH 4 and lower as compared to solo zein fiber mats. However, traces of trifluoroacetic acid (TFA) have been detected, which might have influenced the antimicrobial activity. TFA carries strong carboxylic groups, but the authors argued that the cationic glucosamine groups of chitosan might be the reason for this antimicrobial property and not the TFA itself. Residual solvents were also observed in previously electrospun biopolymers [35]. For food applications however, the presence of residual TFA limits the use of the fiber mats due to the acid's toxicity. *Chitosan acetate.* Quite a number of recent advances of chitosan involve its water-soluble acetate derivative. Like chitosan, the acetate derivative possesses antimicrobial properties. Chitosan acetate can be prepared either by spray drying [36] or by the solid-liquid reaction of chitosan and acetic acid [37], but the latter is preferred for its ease and low cost. This solid-liquid reaction has been used to develop a chitosan coating that was tested on sweet cherries (*Prunus avium* L.). The cherries were used as a model food system since they easily rot at normal fruit storage conditions. A 3-10g/L chitosan acetate solution was found to impede the loss of water, ascorbic acid and titratable acidity and 3-5g/L solutions were enough to extend the postharvest life of the cherries up to 40 days [37].

Besides fruits, chitosan acetate films have also been tested for their antimicrobial effects in fish soup – a more complex food matrix [38]. The films can be prepared by casting a solution of chitosan (low MW, 90.8%DD) with 1% acetic acid. Before addition to the complex food system, it was first tested on *L. monocytogenes*, *Salmonella* spp and *S. aureus*. Bacterial growth reduction was observed for all three tested microorganisms after exposure to the films. The films were then added into test bottles containing the fish soup and allowed to incubate overnight at various temperatures. Interestingly, after the addition of films to the food sample, sensory evaluation was performed to evaluate for consumer liking, food appearance, aroma and flavor. Fernandez-Saiz *et al.* [38] found that the films had reduced effectiveness when added to the fish soup; however, results from the sensory evaluation revealed that the sensory properties did not change. This indicates that further studies are needed to improve the antimicrobial action of chitosan films in a complex food matrix. Though laboratory tests demonstrating its action against test microbes are favorable, this might not be the case for a complex food system that has various components that impedes chitosan's action. The presence of various functional groups in carbohydrates, proteins and other chemicals in food preferably binds to chitosan more than to microbial cells.

3.7.2 Cellulose

Cellulose, the most abundant biopolymer found in nature, has been constantly studied for various applications, mainly on packaging and textile production. In the food industry, cellulose finds applications as a major component in paper or cardboard packaging materials and as thickeners and stabilizers in food. Cellulose and its derivatives are also used as filters or absorbent materials.

Bacteriocins. Cellulose is often utilized in antimicrobial packaging systems as a carrier material for bacteriocins. Bacteriocins are peptides produced by bacteria to inhibit the growth of another closely-related bacteria or strain. Commercially, they are utilized in the medical field and as antimicrobial agents or preservatives of food products.

Nisin, for example, has been added to cellulose films to control the growth of *L. monocytogenes* in processed meat products [39]. Nisin is a known polypeptide harvested from *Lactococcus lactis* subsp. *lactis*. A known and approved food preservative, nisin exhibits optimum antimicrobial action as long as it is not in contact with complex food systems. Its antimicrobial property is sensitive to the presence of other food components resulting in the loss or reduction of antimicrobial activity. Research efforts are geared towards keeping nisin protected by encapsulation or incorporating it in a carrier matrix. Nguyen *et al.'s* [39] cellulose-nisin films demonstrated significant ($P>0.05$) reduction of *L. monocytogenes* growth in processed meat that has been stored at refrigerated conditions. MIC of nisin against *L. monocytogenes* was 312 IU/mL and its incorporation into cellulose films resulted to a MIC value or 625 IU/mL. Incorporation into the film clearly reduced the antimicrobial activity of nisin, but on the other hand, improved the antimicrobial packaging property of the cellulose film. Furthermore, this leaves room for more work on improving the action of nisin that is incorporated in cellulose or any biomaterial. Future research opportunities may investigate the migration of nisin from the film materials to the food product and how its activity is affected during such processes.

Nisin was also added to films made from hydroxypropyl methylcellulose (HPMC), a cellulose derivative. Glycerol was added to the HMPC-nisin film to serve as a plasticizer to improve the material's mechanical property. The HMPC-nisin-glycerol films exhibited antimicrobial effects against *Listeria, Enterococcus, Staphylococcus* and *Bacillus* spp. Mechanical tests revealed that the films have reduced tensile strength but have increased ultimate elongation.

Nisin is not the only antimicrobial additive that was recently added to cellulose films. Pediocin (ALTA® 2351), another antimicrobial peptide, from *Pediococcus* sp, was investigated by Santiago-Silva *et al.* [40] for its activity in a cellulose emulsion against *L. innocua* and *Salmonella* sp. Using processed meat products, cellulose films containing 25% and 50% pediocin were placed on sliced ham that had been immersed in the bacterial solutions. Incubation was carried out under vacuum and at 12°C for 0-15 days. The team found the 50% pediocin-containing cellulose films reduced the growth of *L. innocua* by 2 log cycles after 15 days of incubation. Reduction in *Salmonella* sp. however was low at 0.5 log cycle after 12 days for both 25% and 50% pediocin concentrations [40].

3.8 Other Developments in Active Packaging: Lipid Barrier

Apart from developments in antimicrobial films, a few studies have also reported on the use of biopolymers as lipid barriers [41]. The ongoing interest in chitosan as a potential coating that can limit fat transfer stemmed from results of nutritional studies which had indicated its capability to bind negatively charged molecules [42]. Such molecules can be lipids and fats in the digestive tract, which explains chitosan's popular use as a dietary supplement for weight reduction [43]. This, and the existing campaigns to use biodegradable and renewable materials for packaging, encouraged more inquiries into chitosan's potential coating applications.

Previous studies attributed chitosan's oil barrier capacity to the electrostatic interactions of the protonated amino groups of the polymer, and the negatively charged species of the lipid under a slightly acidic pH range [44]. However, recent studies demonstrated other factors can contribute to these interactions. If electrostatic interactions primarily affect chitosan's binding ability, then it should be influenced by the pH. Chitosan has a pKa~6.5. Consequently, it is soluble and in protonated form ($-NH_3^+$) at pH~5, allowing electrostatic interactions. At basic pH, chitosan precipitates into the solution and becomes uncharged.

Ham-Pichavant *et al.* [45] demonstrated the pH-dependence of a chitosan-oleic acid coating by measuring the free oleic acid residues formed after mixing chitosan with the lipid at various times and concentrations. Oleic acid is a fatty acid molecule with a pKa close to chitosan (~4.8). In a slightly acidic solution, it would carry a negative charge ($-COO^-$) that can bind to $-NH_3^+$ of chitosan. The chitosan-oleic acid coating was observed to be only pH-dependent at pH 5.5-8. Below this, oleic acid is uncharged and does not bind and hence lipid recovery is expected to increase. However, the lipid concentrations recovered were not as high as expected – suggesting other possible interactions taking place. Ham-Pichavant *et al.* [45]_ENREF_60 suggested that emulsion structure and surface properties also influenced binding.

3.9 Food Packaging: Synopsis

Biopolymers are excellent materials for food applications because they are renewable, ecologically-friendly, biodegradable, biocompatible, nontoxic and edible materials. Initial investigations on their use in active packaging, specifically for antimicrobial packaging, show that developing new biopolymer films with increased antimicrobial activity against pathogenic and spoilage microbes is an emerging field in the food processing industry. Though biopolymers are quite a challenge to work with, a few researchers have managed to develop films and coatings that enable the control of bacteria in complex food systems, improving mechanical properties and lipid barrier characteristics.

Future studies can focus on the use of other already approved food additives/biopolymers for food application studies. These may be alginates, gelatin, soy protein isolates and carrageenan. Furthermore, the ability of biopolymers to form complexes and be functionalized or modified for specific applications can be explored.

3.10 Conclusion

Polysaccharides have emerged as an appealing platform for permselective membranes, ionically conductive membranes, food packaging and food additives, because of their renewable nature, biodegradability, biocompatibility, and facile modification pathways. While there are major challenges to working with polysaccharides, there are major advantages as well. Plentiful amine and hydroxyl functionality leads not only to further modification, but to innate antibacterial properties. High levels of functionality paired with strong hydrophilicity, good film-forming character, and excellent chemical resistance properties combine to make polysaccharides very attractive in membrane and film research. Given the successes thus far, the rising international need for fresh water, portable power sources, and fresh food will continue to drive innovation in polysaccharide research.

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4

Starch as Source of Polymeric Materials

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Abstract

Starch is the major carbohydrate reserve in higher plants and has been a material of choice since the early days of human technology. Recently starch gained new importance as a raw material in the production of plastics, in particular, for the synthesis of monomers to produce polymers such as poly(lactic acid) and, after chemical modification (e.g. esterification) and thermomechanical processing, to produce thermoplastic starch. This chapter gives a general overview of the most recent research on the development of materials from starch, focusing on thermoplastic starch and the perspectives for future development in this field. A brief review on reactive extrusion of thermoplastic starch is also provided.

Keywords: Starch, thermoplastic starch, biodegradable polymers, reactive extrusion

4.1 Introduction

Starch is a polysaccharide produced by most higher plants, consisting of D-glucose residues linked predominantly by α-(1, 4) glucosidic bonds. In its native state it has a granular structure composed partially of crystalline regions and insoluble in cold water. Granules can be isolated from seeds, roots, tubers, leaves and fruits. They are formed into spheres, polygons, platelets and others, from 0.5 to 175 μm in size, depending on the species from which they are extracted [1]. Starch is the end product of carbon fixation by photosynthesis and the main carbohydrate reserve in plants. It is thus the main form of carbohydrate food and the principal component in crops such as wheat, maize, rice, barley, potato, cassava and other grain and root crops. However, its use in non-food applications, which includes materials and energy, is of great importance since it is estimated that these industries use more than 20% of the starch (approximately 50% in Europe) produced, a fraction that continues to grow. The growing need for non-oil-derived materials makes starch a strategic material, since it is one of the most efficient products of carbon fixation and

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (81–98)

energy converters in nature. In comparison with cellulose, which is the most abundant natural polymer, starch has the advantage of being easily separated into a very pure form owing to its granular structure. The importance of starch in non-food technological applications dates from the beginning of industrial development in products such as glue for paper and wood, and gum in the textile industry [2-4].

Starch can be obtained in a great variety of crops, the choice of the botanical resource depending mainly on geographic and climatic factors and on the desired functional properties of the extracted starch [5]. It is always possible to find a highly productive plant to produce starch whatever the climate and agricultural conditions, such as maize in temperate and subtropical zones, cassava (the same as manioc or tapioca) in tropical regions, rice in inundated areas, and wheat or potatoes in temperate and cold climates. The main plant sources are maize, rice, wheat, potatoes and cassava [6]. In the year 2005, worldwide starch production accounted for approximately 58 million tons.

Starch is certainly one of the most versatile, inexpensive and readily available materials for potential use in polymer technology. Among the possible uses for starch in materials and energy applications are the following:

- Used as filler in other natural and synthetic polymers.
- Converted into chemicals such as ethanol, acetone and organic acids, used in the production of synthetic polymers.
- Used in the production of biopolymer through fermentative processes.
- Used in hydrolysis to give monomer or oligomer for the production of polyurethanes, for example.
- Destructurized and plasticized starch used as a thermoplastic-like material.
- Chemically modified or grafted with a variety of reagents to produce new polymeris, used as such or as fillers for other polymers.
- Converted by a fermentative process into ethanol for fuel uses.

It should be noted that starch is more readily broken down into small molecules than cellulose, making it an economic option for the production of monomers or as a raw material in the synthesis of other biopolymers. Another advantage over cellulose is the possibility of converting starch to thermoplastic material by physical processing alone, which will be the main topic discussed in this chapter.

The literature concerning starch is vast and its chemistry and technology have been comprehensively reviewed [3, 4, 7, 8]. Renewed interest has arisen in the last decade, more specifically in converting starch into a plastic material capable of replacing petroleum-based counterparts, which is the subject of this chapter. More specifically we will focus on the utilization of starch after destructuration by extrusion processing in the presence of plasticizer and discuss the drawbacks and new perspectives of this emerging technology.

4.2 Starch Structure

Native starch is composed of two main macromolecular components, namely amylose and amylopectin [9-11]. These molecules can be considered homopolymers of anhydrous glucose (α-D-glucopyranosyl moieties) (mol wt = 162 g/mol) formed by its condensation. The monomer residues are linked by (1→4) and (1→6) bonds [9]. Amylose is a predominantly α-(1→4)-D-glucopyranosyl linear macromolecule with a degree of polymerization (DP) of 1000 – 6,000 while amylopectin is a highly branched macromolecule composed mostly of α-(1→4)-D-glucopyranose units, with α-(1→6)-linkages at intervals of approximately 20 units with high molecular weight and a DP of 300,000 – 3,000,000 [12, 13]. Figure 4.1 shows the chemical structure of both polymers and the schematic architecture of amylopectin, according to Robin and Mercier [14].

Starch can be found in various parts of a plant, such as the seed endosperm, the root, the leaf and the fruit pulp. It is deposited in the form of semicrystalline granules which are insoluble in cold water and resemble spherulites [15] of alternating amorphous and crystalline (or semi-crystalline) lamellae.

The granules have been examined by several techniques: light microscopy, electron microscopy, X-ray and neutron scattering and, more recently, atomic force microscopy [16-18]. Starch granules from different plant species are significantly different and can, in the majority of cases, be identified by inspection under a light microscope. The most obvious differences between starch granules are in their shape and size, which vary considerably [2, 4]. Starch granules also contain other minor components, such as lipids, proteins and inorganic compounds, which influence their properties.

The granule grows radially from the *hilum* in alternating amorphous and semi-crystalline rings, forming a lamellar structure [10, 19, 20]. The idealized model describes the crystalline and the amorphous amylopectin lamellae in terms of effectively spherical blocklets, whose diameter ranges from 20 to 500 nm [21].

The amylopectin, which in general comprises around 75% of the granule, is responsible for most of the granule crystallinity. The crystalline and semi-crystalline lamellae are composed of amylopectin blocklets, forming a crystalline hard shell composed of large blocklets and a semi-crystalline soft shell composed of small blocklets. The crystalline lamellae are around 9-10 nm thick on average and consist of ordered double-helical amylopectin side-chain clusters, interfacing with the more amorphous lamellae of amylopectin branching regions. The size of the semi-crystalline soft-shell blocklets ranges from 20-50 nm.

Starch shows distinct crystalline structures that, depending on the source, can easily be identified by X-ray diffraction. The three polymorphisms that can be identified are labeled A, B and C [11, 22]. The most commonly observed structures in native starch are A and B, the former being associated mainly with cereal starches, while the latter generally dominates in tuber starches but also occurs in maize starches with more than 30-40% amylose [11]. Structure

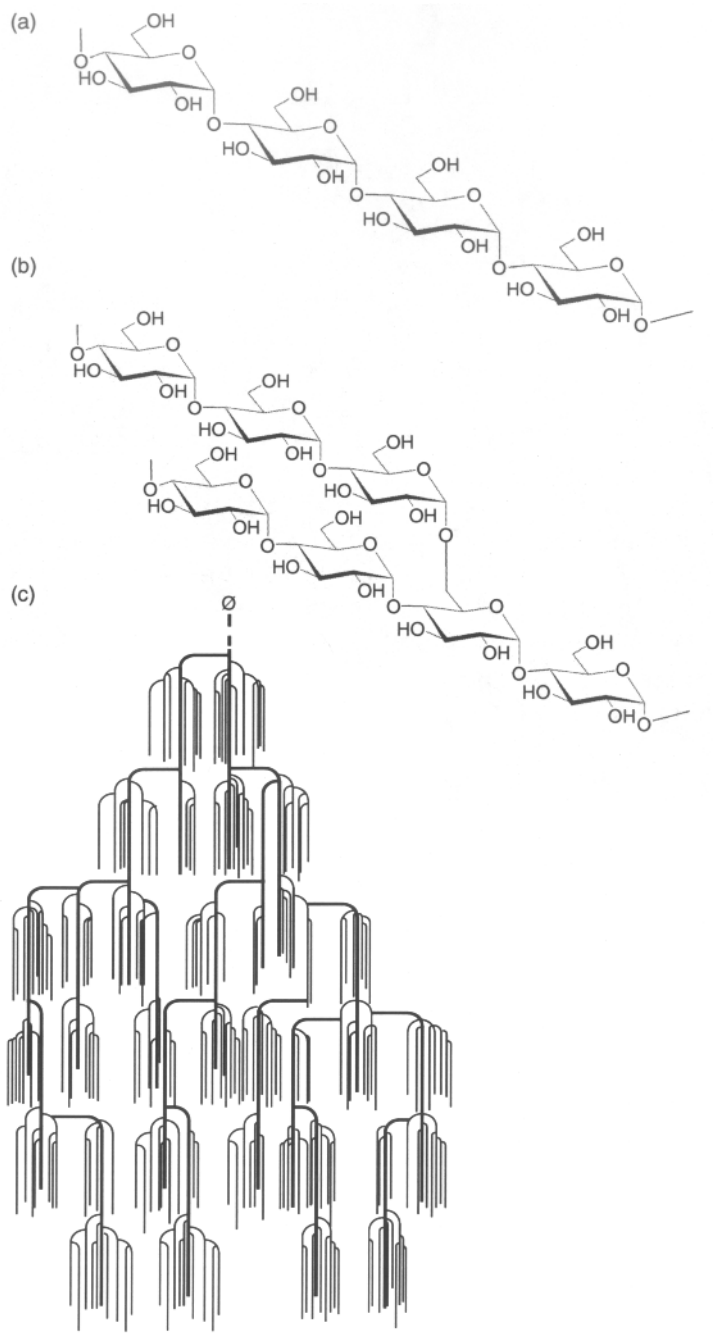

Figure 4.1 Chemical structures of (a) amylose, (b) amylopectin and (c) the architecture of amylopectin, according to Robin and Mercier [14].

C is an intermediate form between A and B and is usually associated with bean and root starches [22, 23]. A-type structure is denser and less hydrated than B-type, whereas C-type arises from the joint presence of the other two

types [1, 11, 14, 22, 23]. Figure 4.2 shows the X-ray diffraction patterns of maize (A-type), potato (B-type) and cassava (C-type) starches.

In contrast to cellulose, whose glucose units are linked by β-1, 4 linkages into linear molecules that form flat ribbons, linear amylose molecules form left-hand helices with the interior mostly lined by hydrogen atoms, resulting in a hydrophobic inner surface [1]. This surface can act as a cover, surrounding other molecules and enabling the formation of complexes with nonpolar linear chains such as the nonpolar tails of lipid molecules (Figure 4.3). The complex of the helix with iodine is responsible for the intense blue colour formed when amylose is mixed with iodine solution [9, 24].

The helical inclusion amylose complexes are called V-structures (Verkleisterung) which are commonly formed by a left-handed amylose helix with six residues per turn enclosing the aliphatic tail of a lipid in its centre [24], each revolution around the lipid taking 8 Å along the axis [25]. V-complexes are insoluble in water, even under severe pressure and temperature conditions, a property that makes them very important in polymer blending when water resistance is the aim [26, 27]. Each glucopyranosyl residue is 4 Å long but in a V-complex each residue occupies 1.33 Å in the axial direction. For the

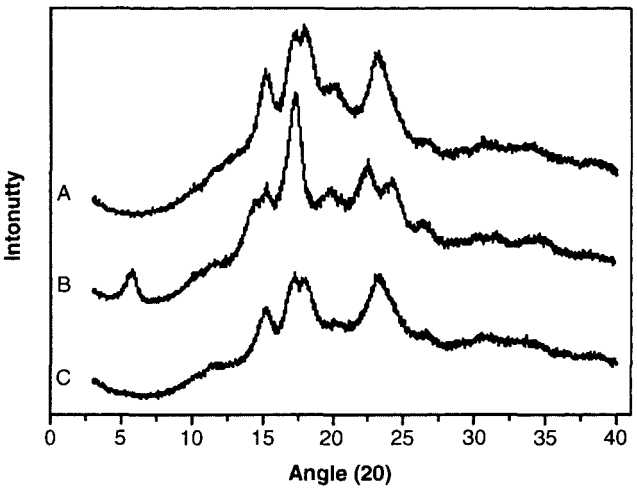

Figure 4.2 X-Ray diffractograms characteristics of A-type, B-type and C-type starches.

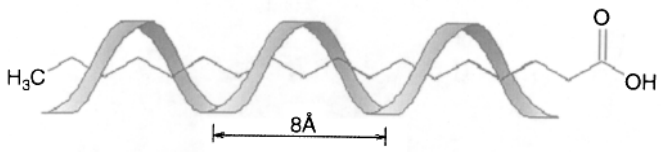

Figure 4.3 Sketch representation of amylose-lipid complex with a C_{18} saturated fatty acid chain. Amylose surround the fatty acid chain forming a helix with the hydrogen atoms oriented inwards. Each turn of the helix (8Å) has six anhydroglucose units [24].

typical strand in the double helix model, amylose is described as an extended structure, with 6 glucose residues that repeat in 21 Å, each residue occupying 3.5 Å in the axial direction [1].

Amylopectin is responsible for the crystalline character of the starch granule and its structure can be modelled as a hyperbranched molecule [10, 11, 28, 29]. The model for the amylopectin molecule proposed by Robin [14] is illustrated in Figure 4.1(c). The A-chains, which are short segments of 15 D-glucopyranosyl residues, are the portion responsible for the crystalline structure of amylopectin. Starch crystallites are thus formed by compact areas made up of A-chains with DP 15. The crystallinity index of regular corn starch with 73% amylose is equivalent to that of waxy maize starch which is 100% amylose; this confirms that amylose content has little effect on granule crystallinity. Starch crystallites formed in compact areas are made up of vicinal A-chains in a compact double helix conformation with two extended helices possessing 6 residues per turn that repeat every 21 Å [1].

The accepted amylopectin model suggests that amylopectin is a clear-cut example of a natural dendrimer [28] with a high degree of branching and a spherical shape, each generation being fully generated from branching sites with a minimum functionality of three.

4.3 Non-food Application of Starch

Starch has been extensively used for several non-food applications, in native state or modified by physical and/or chemical processes. The main areas for starch applications are in adhesives [2], paper [2, 3], drilling fluids for petroleum extraction [30], textile sizing [3], binder in medicines, and hot melt adhesive compositions [31-33].

The other major use of starch is as a raw material for the production of chemicals for the synthesis of other polymers. The production of other chemicals from starch was recently reviewed by Robertson *et al.* [34], Koutinas *et al.* [35], Kennedy *et al.* [36] and Otey and Doane [37]. In general, three different approaches have been employed in the production of polymeric materials from starch-derived chemicals:

1. As a raw material for the production of monomers used in the synthesis of polymers, to replace oil-derived chemicals, such as polyethylene which can be derived from ethanol obtained by fermentation of starch; or of lactic acid, used in the production of poly(lactic acid) (PLA) [38].
2. As a raw material for the production of biopolymers such as polyhydroxyalkanoates (PHA), produced directly from starch by fermentative processes.
3. As a raw material for the production of glucose, dextrin and other hydroxyl-containing monomers.

These processes lead to polymers that are totally different from the starting material, which can be considered a basic chemical raw material.

The disadvantage of using starch as a raw material in comparison with oil-derived chemicals is the cost. Consider, for example, the conversion of starch into polyethylene via its fermentation to ethyl alcohol and subsequent dehydration of the latter [37], the maximum yield of ethylene produced from starch being close to 35%. However, the increase in oil prices and the ecological and political concerns associated with the oil-based economy could in the near future make starch a viable raw material along with other crops such as sugarcane and cellulose from wood and agricultural residues.

Currently, materials derived directly from starch by physical processing or by continuous chemical modification, as in reactive extrusion, are considered the most promising options for bio-based plastic production, particularly on economic grounds. A brief historical view of such starch-based materials is offered in the next section.

4.4 Utilization of Starch in Plastics

Starch utilization in plastic and rubber compositions began in the 60s and 70s, with oxidised starch in rubber and other polymers, such as urethane foams, poly(vinyl alcohol) and copolymers of poly(ethylene-co-acrylic acid) formulations, and as a filler in plasticized polyvinyl chloride (PVC) [37, 39]. In another technique, gelatinized starch was mixed with PVC latex and the water was removed to give a PVC-starch composition, which was mixed with a PVC plasticizer such as dioctyl phthalate (DOP).

Dried starch granules in their native form were used in 1972 by Griffin [39] as a particulate filler for low-density polyethylene (LDPE) in extrusion-blown LDPE films with paper-like texture and appearance. To avoid film defects caused by water volatilization, it was necessary to dry the starch intensively and use special storage, which made this product expensive and of limited use. To increase the adhesion of the hydrophilic starch granules to the highly hydrophobic polyethylene, the granule surface was modified by treating it with reactive silanes, adding extra cost to the process.

In the 70s and 80s, LDPE-starch blends seemed an interesting way of reducing the biodegradability of plastic films which was becoming a big problem, but starch granules were encapsulated into the LDPE matrix and thus became inaccessible to biodegradation. Later studies carried out by Griffin [39] demonstrated that, even when encapsulated, starch could be degraded in an appropriate environment. To increase the rate and extent of biodegradation, a further development emerged with the use of a pro-oxidant such as Fe^{3+} or Mn^{3+}, in the polyolefin matrices such as LDPE [40]. In all of the above-described uses of starch, the granule was used without destruction of its crystalline structure and so starch could be considered a filler in synthetic polymers.

The use of starch as a polymer in blends with other polymers, where the granule structure was modified, increased with the work of Otey and his co-workers [37], who used gelatinized starch with water-dispersible polymers such as poly(ethylene-*co*-acrylic acid) (EAA), in compositions with starch contents of 90% (w/w). These blends were produced by casting the mixture of gelatinized starch and EAA dispersions in water, or by dry milling in a rubber mill, followed by a hot roll treatment, but only the cast films attained acceptable properties [37, 41]. These materials were intended to be used as mulch films for agriculture. Similar techniques were used in the production of starch-EAA blend films by extrusion-blowing, with starch contents between 10 and 40% and EAA between 10 and 90%. In some compositions polyethylene was added in proportions up to 50%. Plasticizers for starch, such as sorbitol, glycerol, urea, starch-based polyols or mixtures of these materials, were also added, with very good results [42]. Urea was used to improve the gelatinization of starch in compositions with low added water and a polyol were added to increase the level of biodegradable material in the final mixture. The main use of these films produced by extrusion-blowing was in mulching applications in the cultivation of various crops. The limited biodegradability and low resistance of these films to the conditions of use limited their usefulness.

The introduction of gelatinized starch expanded considerably the use of starch as a polymeric material, but this technology based on water processing was limited by the necessity of more than one step, some of which were discontinuous. Nonetheless, these developments showed that it was possible to produce blends of synthetic thermoplastic materials with gelatinized starch, affording new prospects of the utilization of starch as a plastic material in the production of films and injection-moulded goods.

The extrusion of starch with relatively low water contents (15 – 25% w/w) was extensively studied by the food industry [43]. One of the first commercial products of starch that achieved very good results was the expanded starch used for protection and as a shock absorber in packages, as a replacement for expanded polystyrene. These materials were patented by the National Starch & Chemical Investment Corporation [44].

At the end of the 1980s, starch processed directly in melting equipment, such as extruders, was described as a new material designated destructurized starch or TPS for ThermoPlastic Starch [45, 46, 47]. This material was patented by the Warner-Lambert Company and was described as being prepared with starch that had been heated to a high enough temperature for a sufficient time for melting to occur prior to starch degradation [48]. In this process, the starch processed in extruders contained between 5 and 40% of water. The process was characterized by an endothermic peak observed by differential scanning calorimetry (DSC), before the decomposition temperature, which did not appear in the processed material. It was also claimed that when starch was heated in a sealed volume in appropriate moisture and temperature conditions, it became substantially compatible with hydrophobic thermoplastic synthetic polymers.

A different approach was used by the Ferruzy Company, the main difference being the use of high boiling-point plasticizer instead of water for the destructuration of starch. In this technology, starch was plasticized together with polymers such as polyethylene-vinyl alcohol (EVOH), EAA, poly-ε-caprolactone, with small amounts of moisture, in a twin-screw extruder [49], to produce an intimate mixture between starch and the synthetic polymer. The commercial trade name of this product family is Mater-Bi^{TM}.

Owing to the method and the type of plasticizer used for TPS production, the process leads to the destruction of the starch granule lamellar structure, giving rise to a quasi-amorphous or semi-crystalline material.

4.5 Some Features of the Physical Chemistry of Thermoplastic Starch Processing

Here we will briefly discuss two major topics related to thermoplastic starch physical chemistry: the starch granule destructuration or gelatinization during TPS production and the macromolecular properties of the semicrystalline plasticized starch which include indistinctly thermoplastic starch, destructurized starch and plasticized starch.

The physicochemical properties of starch that are most important to thermoplastic starch production are those related to its gelatinization and/or destructuration. Starch granules ranging from 0.5 – 175 μm in diameter are insoluble in cold water and partly crystalline, which allows their isolation and purification. The processing of starch is generally conducted in excess water, in which the granules swell as the temperature is increased, leading to their progressive hydration and partial solubilization mainly of amylose which is progressively leached from the granules. The process can be broken into two steps: hydration or diffusion of the solvent through the granule, followed by the melting of the starch crystallites.The latter process is named gelatinization, which involves loss of order in the granule and consequently of its birefringence and X-ray crystallinity. Solvents other than water, such as liquid ammonia, dimethyl sulfoxide, organic acids, sugars and alcohols promote or affect the gelatinization by disrupting hydrogen bonding within the granule [50]. From a thermodynamics point of view, gelatinization can be described as the melting of starch granules. Gelatinization occurs above a characteristic temperature known as the gelatinization temperature.

Regarding starch granule plasticization, we will focus our discussion only on the thermal transitions involved and some considerations regarding the loss of order during this process.

The order-disorder transition occurring during gelatinization or destructuration has been studied by several techniques, including X-ray scattering, light scattering, optical microscopy (birefringence using crossed polarizers), thermomechanical analysis and NMR spectroscopy and, more recently, small-angle X-ray scattering (SAXS), wide-angle X-ray scattering (WAXS) and small-angle

neutron scattering (SANS) [22, 51, 52]. On the basis of evidence gathered by these techniques, Jenkins and Donald concluded that the final loss of crystallinity only occurs when gelatinization is almost complete [52].

Calorimetric methods such as Differential thermal analysis (DTA) and differential scanning calorimetry (DSC) [15, 52] have elucidated important aspects of starch gelatinization. The work of Donovan [15], who used the DSC technique to analyze starch suspensions in water with various starch:water proportions, showed that a single endothermic peak is produced when the water volume is below ~0.38. Increasing the water volume fraction (volume fraction, $\nu > 0.7$) this peak disintegrates into two endothermic transitions [15]. Donovan studied suspensions of potato starch with volume fraction of solvent varying from 0.81 to 0.28. In this classic study, Donovan [15] demonstrated that at high solvent volume fractions ($\nu > 0.81$), a single endotherm was observed at a fixed temperature characterized as the gelatinization temperature (for a potato 66 °C). This transition had been observed and described in previous works [51]. The enthalpy change associated with this transition was in the range of 3.4 to 5.3 cal/g. At volume fraction $\nu > 0.7$, a shoulder appears at a higher temperature, and as ν is reduced, it grows into a peak at progressively higher temperatures, while the gelatinization peak at 66 °C shrinks, disappearing at ν ~0.38. This behavior is shown in Figure 4.4.

At a volume fraction of water greater than 0.7, which corresponds to a molar ratio of water to anhydroglucose > 14, the largest endothermic peak is seen. Increasing the water fraction from this point does not change the heat of this endotherm. These results show that two distinct processes occur during starch gelatinization.

The high temperature endothermic peak can be treated thermodynamically, because its temperature and intensity are dependent on the solvent volume fraction and independent of the heating rate, as observed by Donovan. This behaviour has been treated quantitatively, using Flory's relationship between the melting point of the crystalline phases and the quantity of added water [15, 45]. This relation between melting point and water content is shown in Equation 4.1 and its equivalent, Equation 4.2:

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \left(\frac{R}{\Delta H_u}\right)\left(\frac{V_u}{V_1}\right)(v_1 - \chi_1 v_1^2) \tag{4.1}$$

$$\frac{\dfrac{1}{T_m} - \dfrac{1}{T_m^0}}{v_1} = \left(\frac{R}{\Delta H_u}\right)\left(\frac{V_u}{V_1}\right)(1 - \chi_1 v_1) \tag{4.2}$$

where R is the gas constant, ΔH_u is the enthalpy of fusion per repeating unit, V_u/V_1 is the ration of molar volumes of the repeating unit and the diluent, ν_1 is the volume fraction of the diluent, χ is the Flory-Huggins polymer-diluent interaction parameter, and T_m^0 is the melting point in the absence of diluents.

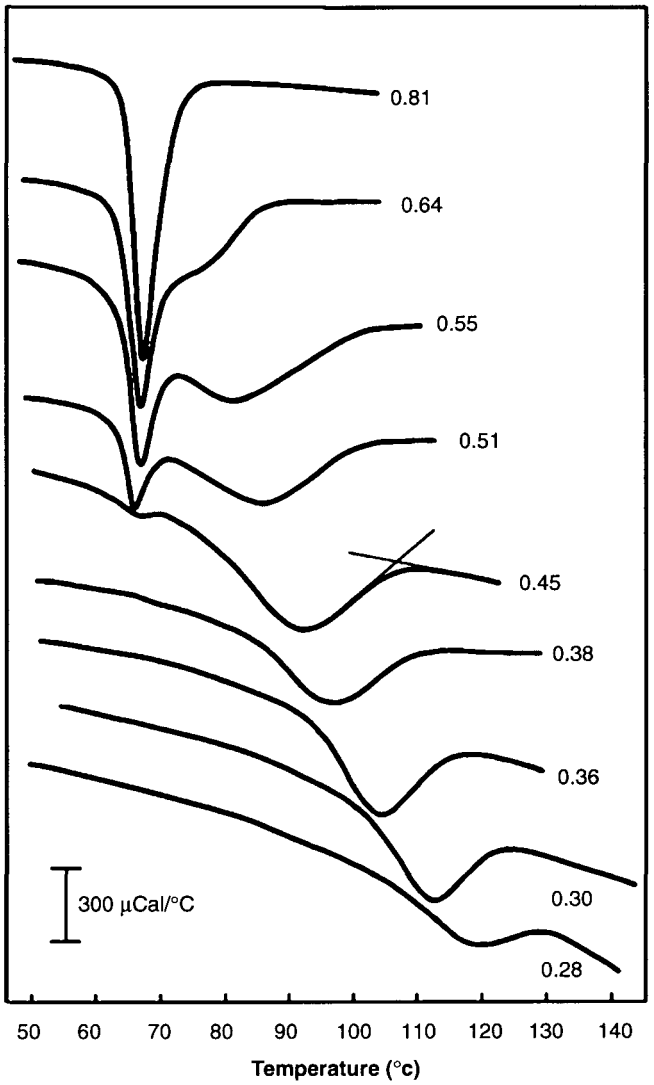

Figure 4.4 DSC thermograms of potato starch-water in sealed pans, recorded at a heating rate of 10 °C/min, with the volume fraction of water varying from 0.28 in the bottom curve to 0.81 in the top curve. Reproduced with permission from ref. [15].

The melting point, T_m^0 is taken as the melting point of the most perfect crystallities. T_m^0 and ΔH_u were determined graphically from plots of $1/T_m$ versus v_1 when $\chi_1 = 0$ (equation 1) and $(1/T_m^0 - 1/T_m)$ against v_1 (Equation 4.2), respectively [15]. The value for ΔH_u and T_m^0 determined for several starches were close to 14 kcal/mol per D-glucose residue and from 168– 197 °C, respectively [15, 50].

The low-temperature endothermic transition is associated with gelatinization and shows no dependence on water content, while being observed in excess water. According to Donovan, this process is caused by the dissolution of individual starch chains by the swelling action of water.

The granule order can also be destroyed in the absence of water, or at very low water concentration, by the action of high boiling point hydroxyl-containing compounds, such as glycerol, glycols and sugars (glucose, fructose and others), which act as plasticizers. This process is distinct from gelatinization and produces an amorphous mass known as destructurized starch or, more widely, as thermoplastic starch. This kind of processing is usually performed in equipment designed for plastic production, the most important and widely used of which are extruders, both single and twin screw.

More recently Li *et al.* have proposed that the process of gelatinization conducted at low moisture content could be defined as the "melting" of starch [53] in accordance with Donovan [15]. Concerning the kind of plasticizer/solvent used, Perry and Donald concluded that during gelatinization or destructuration of the starch granule, the resulting plasticized starch is of the same nature, irrespective of whether the solvent is pure water, glycerol, ethylene glycol or a range of high molecular weight sugar solutions. The only variation observed is in the characteristic initial gelatinization temperature [54].

In an extrusion process, where shear forces and high pressures are applied, the entire process is obviously much more complicated. However, either with limited amounts or in excess of water, the main step associated with the melting of crystallites is the same, leading to an amorphous entangled mass of amylose and amylopectin macromolecules.

Thermoplastic starch recrystallizes after extrusion in the B-type crystalline structure, the crystallinity index increasing with the amypolectin content in the TPS [55, 56]. This process leads to a more brittle and fragile material and several studies have been aimed at reducing this effect. Recently we investigated the dependence of the molecular weight on the crystalline melting point of TPS produced from regular corn starch plasticized with 30 wt% of glycerol. The TPS varying molecular weights were produced by processing the starch in the presence of organic acids, such as citric and ascorbic. In this study, the heat of fusion (ΔH_f) was about 120 J/g and independent of the molecular weight of starch in the TPS. In contrast, the melt temperature, T_m, was dependent on the molecular weight and this was explained by the presence of end-*mer* units at the ends of the starch chains. These end-*mer* groups differ from the *mers* that constitute the chains and act as if they are diluting the TPS. A linear relation between $1/T_m$ and $1/M_n$. was observed, taking ΔH_f as invariant. This behavior was observed only in the TPS modified with organic acids [57].

4.6 Recent Developments in Thermoplastic Starch

Currently, great efforts are being made to produce thermoplastic starch with improved mechanical properties, low water sensitivity and better compatibility with other polymers, which is of fundamental importance to the production of TPS-based blends. Studies have also been carried out on multilayered systems, as an interesting way to overcome some TPS drawbacks [58]. These

topics have been the subject of comprehensive reviews published recently [58-60]. One of the emerging techniques to develop new forms of TPS with great potential is reactive extrusion processing of starch, which in general is used to improve the compatibility of TPS with other polymers. This area of research is of great importance because new blends of TPS, which could not be attained without some chemical modification, can be made to enable, for example, the production of compatible blends of TPS and polyethylene. Here we will focus the discussion on the reactive extrusion of TPS as one of the tools for the development of new materials based on TPS

4.7 Reactive Extrusion

In reactive extrusion (REX), chemical reactions take place during the processing of the melt. The reactant can be added at several stages of the process; it is possible, at the same time, to remove volatile by-products of the reaction and to introduce reactants at points along the extruder as well as combining zones of melting, transport, low pressure and high pressure/shear zones. REX is mainly used for polymer modification, however, polymerization itself can also be performed [61] to produce high polymers or oligomers. In this case, the extruder operates as a continuous reactor, enabling the processing of high viscous materials without a need for solvents [61, 62]. Several kinds of reaction can be produced by REX, such as, i) polymerization of monomers or oligomers via free radical, condensation or coordination, ii) degradation or depolymerization, iii) cross-linking , iv) functionalization of conventional polymers, v) grafting and others [61, 62].

The use of REX for the modification of TPS has been reviewed recently by Xie *et al.* [63], Kalambur and Rizvi [64, 65], and Raquez *et al.* [66]. The reactive blending of thermoplastic starch has been successfully used for TPS compatibilization with several polymers [63, 64, 67-69]. Examples of reactive blending of TPS have been described recently for PLA [68-72], PHB [73, 74], polyethylene [74-77], polyurethanes [78] and polyesters [79, 80].

Reaction with vinyl acetate [81], hydroxypropylation [82], reactions with styrene [83], with ethylene glycol and other glycols giving rise to glucosides [63, 84] or with acrylamide monomer [85, 86] have also been described. Reactive extrusion is also used to decrease the melt viscosity and decrease the interfacial tension of TPS-based blends [76]. Ning *et al.* [74] studied the effect of adding citric acid on TPS and LLDPE, via a single-step reactive extrusion. The authors showed improvements in the compatibilization and the mechanical properties and shifts of polyethylene peaks observed by FTIR.

Miladinov *et al.* reported the preparation of starch-fatty acid esters by reactive extrusion of plasticized starch and acid anhydrides (acetic, propionic, heptanoic and palmitic anhydrides) in the presence of sodium hydroxide as a catalyst [87]. Starch esters have been prepared by REX using maleic anhydride (MA) as a cyclic dibasic acid anhydride in the presence of 20 wt% glycerol as plasticizer. This material was melt-blended with biodegradable polyester,

forming compatible blends. Some hydrolysis and glucosidation reactions by MA moieties were reported [79].

Shujum *et al.* described compatible TPS/LLDPE blends, produced by one-step reactive extrusion in a single-screw extruder. Maleic anhydride (MAH), and dicumyl peroxide (DCP) were used to graft MA onto the LLDPE chain [88].

A maleated ester of low density polyethylene, prepared by the reaction of LDPE and dibutyl maleate in solution, was blended with TPS by reactive extrusion. Mechanical properties of the blends were similar to those of the LDPE due to the compatibilization of these dissimilar components [75].

Huneault and Li described compatible blends of TPS-PLA made by free-radical grafting of maleic anhydride (MA) on the PLA backbone and then by reacting the modified PLA with the starch macromolecules in a twin-screw extruder provided with a degassing zone to eliminate water and free unreacted MA at both the PLA grafting and TPS-PLA blending steps. MA-grafted PLA showed a much finer dispersed phase, in the 1–3 μm range, and exhibited a dramatic improvement in mechanical properties [69].

The compatibilization of TPS and PLA has also been achieved in a one one-step process described by Wang *et al.* Dicumyl peroxide and maleic anhydride (MA) were used. The process was conducted in a single-screw extruder by one-step reactive extrusion [72].

Cationic starch has been prepared in the molten state by the reactive processing of TPS plasticized with 15% w/w glycerol using sodium hydroxide as the catalyst and 3-chloro-2-hydroxypropyltrimethylammonium chloride and 2-epoxypropyltrimethylammonium chloride as cationization reactants [89].

Compatibilization by reactive extrusion of starch and other polymers such as PLA has also been carried out successfully with diisocyantes such as 4,4-diphenylene diisocyanate (MDI) as reagent [80, 90].

We recently introduced a new approach, depolymerization of TPS followed by a polymerization step, in a single-step reaction. The depolymerization was conducted with citric acid, producing partially esterified starch plasticized oligomers. The polymerization with a di-functional isocyanate was conducted in the same step, but at the higher temperatures found in the front zone of the extruder. Single-step processing represents a simple and viable method for the production of new low-cost biodegradable polymers. The possibility of controlling the process by the two independent reactions allows a large range of TPS to be produced with tunable physical and chemical properties. In the depolymerization reaction the extent of chain breaking was controlled, while in the re-polymerization, the extent of polymerization and the type of group formed during the process were under control.

4.8 Conclusion

The fast growth in emerging new technologies for starch utilization as a thermoplastic material, such as multi-layered composites, nanocomposites

and reactive extrusion, will lead to a vast expansion of new starch-based products that will have the potential to replace several synthetic commercial polymers. These technologies show at least two fundamental characteristics from the point of view of the market, i) they allow the designing of continuously tunable processed materials and ii) on a larger scale of production, they are low-cost, due to the inexpensive raw materials and to the use of standard processing tools, largely available in the plastics industry. These technologies use starch as a source of a polymer or oligomer in place of a simple raw material. TPS does not need to have the same characteristics as commercial synthetic polymers such as polyethylene and polyesters, but the ability to control its properties and characteristics opens up the possibility of producing compatible blends with those polymers and creating a niche TPS in the market.

The emergence of TPS with tunable properties, by using continuous reactive extrusion, can lead starch to a new position in the market of thermoplastic materials based on renewable resources with functional properties such as biodegradabitly.

Acknowledgement

The author wishes to thank the State of São Paulo Research Foundation (FAPESP) and The National Council for Scientific and Technological Development (CNPq) for the financial support for starch related projects in which the author is involved.

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5

Grafted Polysaccharides: Smart Materials of the Future, Their Synthesis and Applications

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Abstract

Polysaccharides are the most abundant natural organic materials. They are known for several versatile applications; however, modulation of their significant properties towards their industrial perspectives can be achieved by grafting techniques. Microwave triggered grafting techniques are the most convenient and efficient methods to prepare grafted polysaccharides. The high control over the hydrodynamic volume of the grafted polysaccharide macromolecule makes it an ideal material as a flocculent for water/wastewater treatment and for rheological applications such as a thickener/viscosifier. Furthermore, a polysaccharide-based drug delivery matrix can be programmed at the molecular level, using grafting techniques, to precisely control the rate of release of the enclosed drug. Thus, grafted polysaccharides can play a central role in drug delivery research. Evidently, the tailor-made grafted polysaccharides have the potential for success in the areas of waste water treatment, rheological applications and drug delivery research.

Keywords: Ceric ammonium nitrate, controlled drug release, flocculation, microwave assisted grafting, molecular programming, polysaccharide, SEM, intrinsic viscosity, rheology, drug delivery system

5.1 Introduction: Polysaccharides as a Material of the Future

Polysaccharide materials are the most abundant and diverse molecules present as an integral part of the natural system on this planet. They are renewable, biodegradable and can be cultivated as plant biomass, which is essentially made of polysaccharides. Nature has been using polysaccharides from time immemorial. Polysaccharides serve as structural material (e.g. the cell walls of plant cells), and are used for energy storage in the form of starch in plant cells and as glycogen in the liver cells of animals.

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (99–128)

Since the beginning of time, polysaccharides have been of immense importance to human civilization. They are a vital component of our food as an energy source (e.g. starch), and also as roughage (e.g. cellulose fibers) which aids in digestion. They are the earliest known structural materials, i.e. in the form of wood (which is chiefly 'cellulose', a polysaccharide), that still hold a prominent place as an engineering material. The role of polysaccharides in human civilization is vast, and they surround us all the time, even in this era of synthetic materials/plastics; in the form of textiles, paper, glue, food and food additives, etc., without causing any major environmental 'disposal' problems (unlike their synthetic counterparts).

Thus, the industrial applications of these natural (unmodified) polysaccharides are huge. So, it is obvious that their chemical modifications, 'tailor-made' towards specific property, can result in new materials with much wider applications for human civilization.

From their vast abundance, renewability and eco-friendly nature, it seems logical that in today's world, suffering from both the impending danger of depletion of non-renewable resources (coal/petroleum/natural gas), as well as an ever increasing 'waste disposal' problem (due to the non-biodegradability of synthetic polymeric materials), modified polysaccharides designed for specific applications would be of immense importance.

5.2 Modified Polysaccharides

5.2.1 Modification by Insertion of Functional Groups onto the Polysaccharide Backbone

One of the effective ways of modifying polysaccharide is by the incorporation of functional groups (e.g. carboxymethyl groups, carboxyethyl groups, etc.) onto its backbone, resulting in new material with modified properties, thereby creating possible diversified applications in new domains as compared to those of the base polysaccharide itself.

A well-known example is the incorporation of a sodium carboxymethyl group onto the cellulose backbone resulting in a profound change in its properties and hence diverse usage (e.g. cellulose is insoluble in water while sodium carboxymethyl cellulose is water soluble and has applications as a viscosifier). The insertion of a carboxymethyl group is a common example of the modification of polysaccharide. The obvious changes are evidenced in solubility, swelling properties, reduced biodegradability (hence, longer shelf life) and anionic nature. In solution, polysaccharides tend to assume coiled conformations due to intramolecular associations (e.g. hydrogen bonding). This results in much less hydrodynamic volume of the polysaccharide macromolecule. The repulsion between inserted carboxymethyl groups (CH_2COO^-) in solution, stretches the polysaccharide backbone, resulting in the opening up of its coiled conformation and hence, a substantial increase in hydrodynamic volume. This increase in hydrodynamic volume opens up the possibility of applications as a flocculant for water/wastewater treatment and rheological applications.

5.2.2 Modification by Grafting of Chains of Another Polymeric Material onto Polysaccharide Backbone

Grafting is the branching of one type of polymer chain (graft) from another type of polymer chain (backbone), as shown in Figure 5.1.

Grafting of polysaccharides and modified polysaccharides indeed consists of an effective method to modify their properties in accordance to our specific needs. The *quantitative regulation* of properties can be achieved simply by controlling the synthesis parameter called 'percentage grafting'. Thus, in other words, one can 'tailor' certain desired properties in a natural/ modified polysaccharide, simply by grafting it with another polymer, in an appropriate percentage of grafting.

$$\% \text{ grafting} = \frac{\text{wt. of graft copolymer} - \text{wt. of polysaccharide}}{\text{wt. of polysaccharide}} \times 100$$

Grafting reactions are mostly free radical reactions [1]. The process of grafting starts with a preformed polymer (backbone) and monomers of the other polymer (to be grafted). Now, free radical sites are essentially created on the preformed polymer. They then initiate a free radical reaction with the monomer, yielding the graft copolymer. Competing homopolymer formation reactions also take place, resulting in a homopolymer byproduct which needs to be separated by solvent extraction to get the pure graft copolymer product.

The free radical sites on the preformed polymer can be created by a variety of ways (Figure 5.2). Conventionally, free radical initiators such as ceric ammonium nitrate (CAN) have been used for this purpose [2–31]. Alternatively, high energy radiations like gamma rays and electron beams or radiations like UV rays can also be used [32–46].

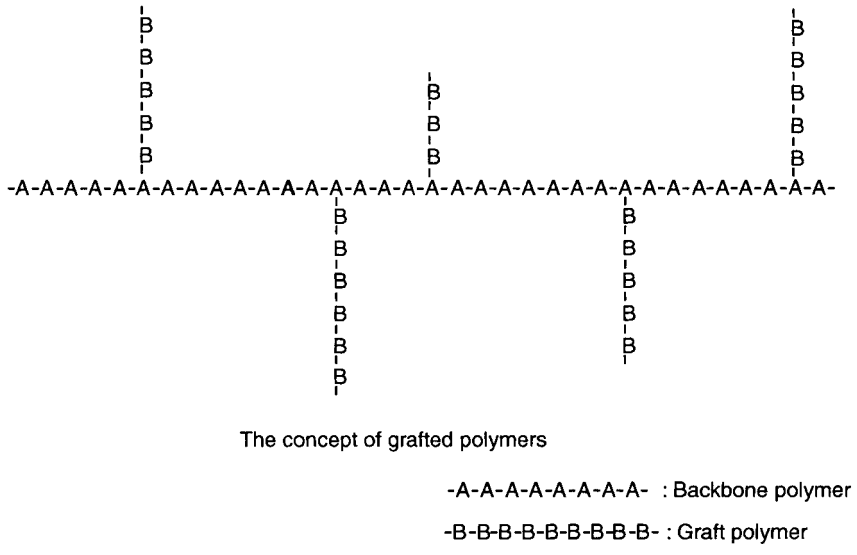


Figure 5.1 Concept of polymer grafting.

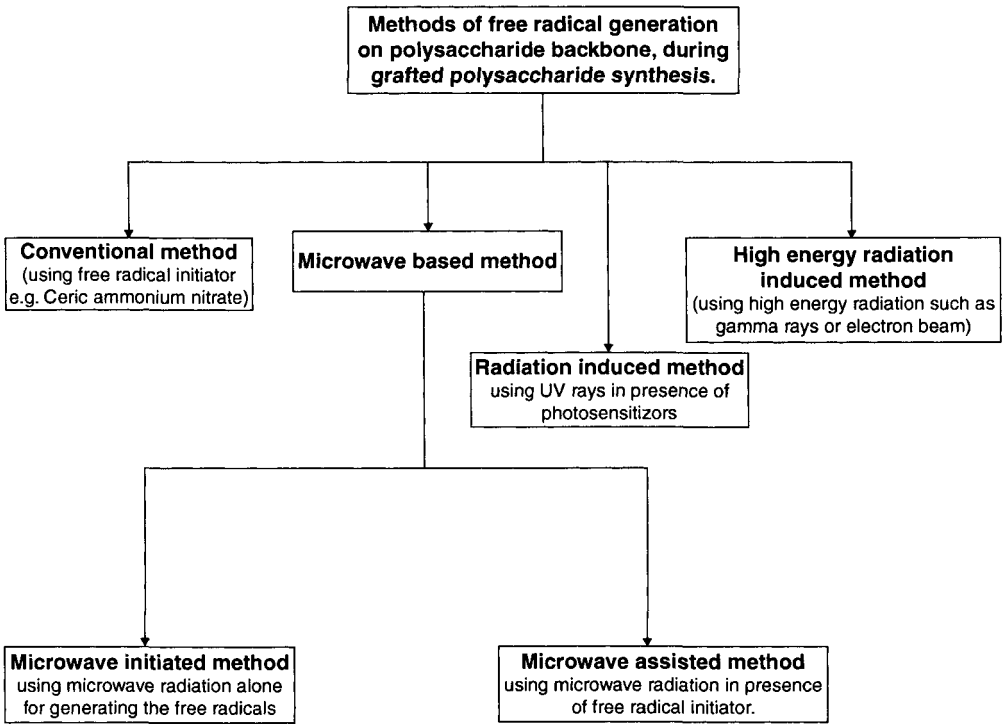


Figure 5.2 Methods of free radical grafting.

A recent approach of generating the free radical sites is by the use of microwave radiation alone [47–51]. This approach eliminates the use of any 'chemical' free radical initiator, thus conforming to the norms of 'green chemistry'. The process is very simple (e.g. no inert atmosphere is required unlike the case of 'ceric ammonium nitrate' initiated grafting) and fast. This makes the microwave radiation-based grafting process a more suitable and highly reproducible method of the synthesis of graft copolymers. This recent method of graft copolymer synthesis is referred to as *microwave initiated synthesis* [51]. In this method of graft copolymer synthesis, the initiating factor is the microwave radiation, whose power and irradiation time are electronically controlled. It is evident that by using this method one would have precise control and high reproducibility of percentage grafting; thus, high batch-to-batch consistency can be expected.

Another method of graft copolymer synthesis is a combination of microwave-based and conventional synthesis, i.e. using both microwave radiation as well as a chemical free radical initiator (e.g. ceric ammonium nitrate) together. This process is referred to as *microwave assisted synthesis* [47]. Although it yields a higher percentage grafting than the *microwave initiated synthesis*, the reliability of the synthesis process is low.

Two profound changes observed due to grafting of polyacrylamide chains (PAM) on a polysaccharide are an increase in hydrodynamic volume and

decrease in solubility of the macromolecule. The former change renders the graft copolymer as an efficient flocculant, while the later change makes it a better matrix for controlled drug release.

5.2.2.1 *Synthesis of the Graft Copolymer by Conventional Method (Using Ceric Ammonium Nitrate as a Free Radical Initiator)*

Grafting reactions are conventionally carried out by using the ceric ion induced redox initiation method [51, 52]. The details of the synthesis and the reaction conditions are as follows:

1 gm of polysaccharide is dissolved in 100 ml of distilled water with constant stirring and bubbling of a slow stream of nitrogen for about 15 minutes. The required amount of monomer for the graft is dissolved in 50 ml of distilled water and mixed with the polysaccharide solution. Then oxygen free nitrogen gas is purged through this reaction mixture for another 40 minutes. At this stage, 10 ml of ceric ammonium nitrate (CAN) solution of catalytic concentration is added and accordingly nitrogen gas purging is continued for another 15 minutes. The reaction is allowed to continue for 24 hours, after which it is terminated by adding a saturated solution of hydroquinone. At the end of the reaction, the polymer is precipitated by adding an excess of acetone. It is then dried in a hot air oven. Subsequently, it is pulverized and sieved. The reaction temperature is maintained at 28±1°C. The reaction parameters in case of synthesis of polyacrylamide grafted carboxymethyl starch (CMS-g-PAM) by this method [51], is shown in Table 5.1.

The most widely used method of chemical initiation for graft copolymerization onto polysaccharides has been with ceric salts such as ceric ammonium nitrate (CAN) or ceric ammonium sulphate (CAS). At low temperature, CAN is more efficient because of its instability at an elevated temperature [53]. The mechanism by which Ce (IV) generates free radicals is believed to involve the formation of a chelate complex between the hydroxyl group of the polysaccharide and the oxidant. The complex so formed disproportionately forms free radicals on the polysaccharide backbone. These active free radical sites in the presence of acrylic monomers generate graft copolymers. The earlier studies based on a model compound using Ce (IV) oxidation of polysaccharides and 1,2-glycols support the above explained mechanism and suggest that the $C_2 - C_3$ glycol and the C_5 hydroxyl groups of D-anhydroglucose unit of polysaccharide may be the preferred sites for free radical generation [54]. Based on the above explanation, the plausible mechanism that has been proposed for the initiation of graft copolymerization by ceric ion is shown in Figure 5.3. A series of five graft copolymers have been synthesized with CMS by the conventional method. For the first three graft copolymers (I-III) (Table 5.1), the catalyst (CAN) concentration was varied, keeping the concentration of acrylamide and CMS fixed. For the second set of three graft copolymers (II, IV-V), only the acrylamide concentration was varied, keeping the other parameters constant.

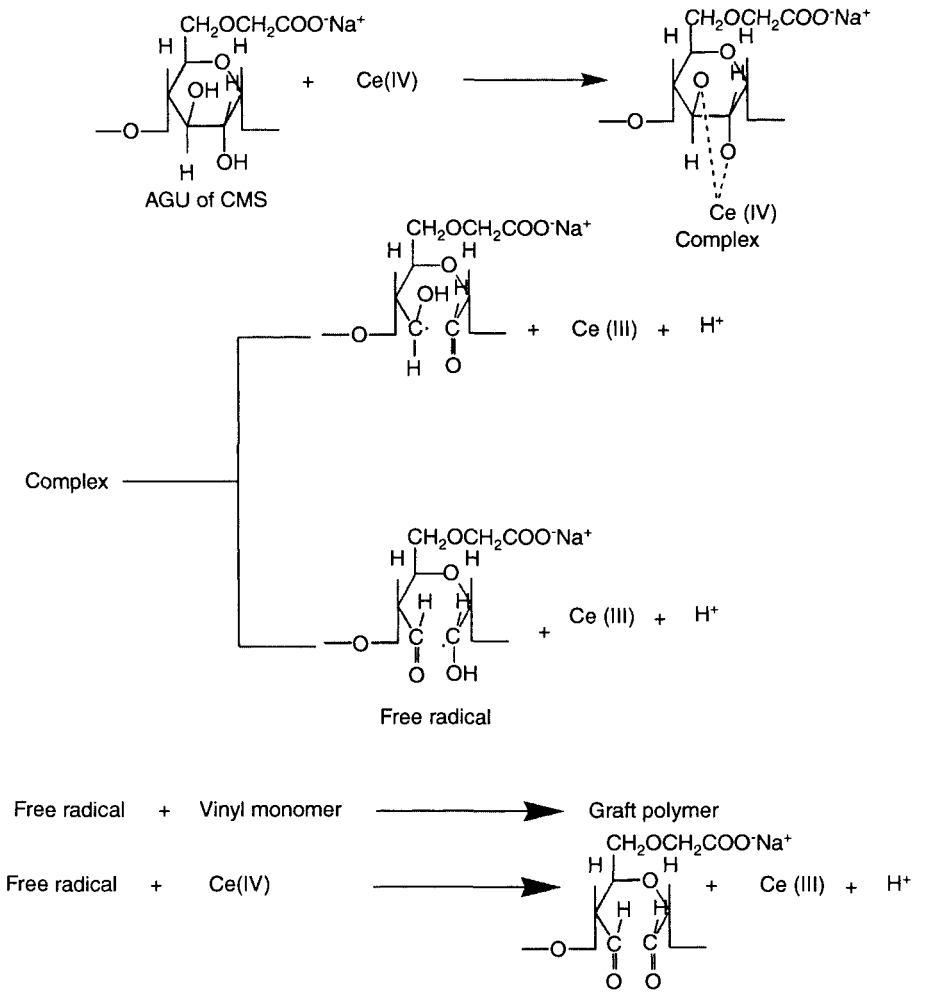
Table 5.1 Synthesis of CMS-g-PAM by conventional and microwave initiated method (reprinted from [51]).

By Conventional Method (Using Ceric Ammonium Nitrate as Free Radical Initiator)						
Polymer Grade	**Amount of CMSa (mole)**	**Amount of Acrylamide (mole)**	**Amount of CAN (mole×10-4)**	**Intrinsic Viscosity (dL/g)**	**%Grafting[b]**	**Wt. Average Mol .Wt (K Da)**
CMS-g-PAM I (C)	0.0062	0.14	0.21	2.6	18%	1160
CMS-g-PAM II (C)	0.0062	0.14	0.42	4.4	28%	1800
CMS-g-PAM III (C)	0.0062	0.14	1.05	4.0	24%	1650
CMS-g-PAM IV (C)	**0.0062**	**0.21**	**0.42**	**6.8**	**47%**	**4300**
CMS-g-PAM V (C)	0.0062	0.28	0.42	6.5	45%	2920
By Microwave Initiated Synthesis (Using 900 W Microwave Radiation)						
Polymer Grade	**Amount of CMSa (mole)**	**Amount of Acrylamide (mole)**	**Time (min)**	**Intrinsic Viscosity (dL/g)**	**%Grafting[b]**	**Wt Average Mol .Wt (K Da)**
CMS-g-PAM I (M)	0.0062	0.07	1	4.02	25%	1720
CMS-g-PAM II (M)	0.0062	0.07	2	5.6	40%	2150

CMS-g-PAM III(M)	0.0062	0.07	3	6.2	43%	2350
CMS-g-PAM IV(M)	0.0062	0.07	4	5.9	41%	2210
CMS-g-PAM V (M)	**0.0062**	**0.14**	**3**	**7.4**	**50%**	**4790**
CMS-g-PAM VI(M)	0.0062	0.21	3	6.7	46%	3240
Carboxymethylstarch (CMS)						
CMS 4	–	–	–	2.06	0%	874

[a]Calculated on the basis of anhydroglucose unit (AGU). 1 mole of AGU = 162 g.

[b] $$\%\text{grafting} = \frac{\text{wt. of graft copolymer} - \text{wt. of polysaccharide}}{\text{wt. of polysaccharide}} \times 100$$



Structure of the grafted polymer synthesized:

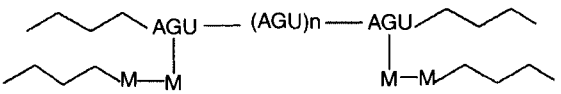


Figure 5.3 Mechanism of ceric ammonium nitrate (CAN) initiated synthesis of polyacrylamide grafted carboxymethyl starch (CMS-g-PAM).

5.2.2.1.1 Effect of Initiator Concentration

When a simplistic approach is followed, a low concentration of catalyst should initiate a few grafting sites, which results in longer polyacrylamide chains, compared to a high concentration of catalyst, which will initiate a larger number of grafting sites, making the average size of grafted chains shorter for the same monomer concentration. So during the grafting of polyacrylamide onto

CMS, there are two possibilities. One can either have a small number of long polyacrylamide chains or a large number of short polyacrylamide chains in the graft copolymer. In the former case, the compact shape of the original CMS would be changed, due to the presence of long polyacrylamide chains. This would result in larger hydrodynamic volume, leading to higher intrinsic viscosity. On the other hand, a large number of short polyacrylamide chains will not alter the original compact shape to a great extent, thus resulting in lower hydrodynamic volume, which should be reflected again in its lower intrinsic viscosity value.

5.2.2.1.2 Effect of Monomer Concentration

With an increase in the concentration of acrylamide (From 0.14 moles – 0.28 moles), percentage grafting increased continuously and achieved the maximum when the concentration of acrylamide was 0.21 moles. Afterwards, the percentage grafting decreased. This behavior can be explained by the fact that an increase in monomer concentration leads to the accumulation of monomer molecules in close proximity to the CMS backbone. The decrease in the percentage grafting after optimization could be associated with the reduction in the active sites on the CMS backbone as graft copolymerization proceeds. It can also be accounted for because once the graft copolymer radical has formed, the excess monomer will shield the graft copolymer, which may decrease the rate of graft copolymerization. In addition to this, with excess monomer concentration, the competing homopolymer formation reaction becomes significant, leading to a depletion in the percentage of grafting.

5.2.2.2 Synthesis of the Graft Copolymer by Microwave-Initiated Method

The microwave-initiated method involves microwave radiations alone as the free radical initiator. There is no requirement for a conventional free radical initiator such as CAN for the grafting process.

Recently, polyacrylamide grafted carboxymethylstarch (CMS-g-PAM) has also been synthesized by this method [51]. Various grades of graft copolymer were synthesized by varying the irradiation time and the monomer (acrylamide) concentration, as shown in Table 5.1. The optimized grade has been determined through its higher percentage grafting and intrinsic viscosity. From Table 5.1 it is obvious that the grafting is optimized at a monomer concentration of 0.14 moles and at an irradiation time of 3 minutes, when the microwave power is maintained at 900 watts.

5.2.2.2.1 A Plausible Mechanism of Free Radical Generation by MW Radiation

When small polar molecules such as water are irradiated with microwave radiation, it results in a rotation of the molecules, leading to the generation of heat. However, no free radical is expected to be produced as such.

However, if bigger molecules or macromolecules are present, only the highly polar groups (O-H & N-H) interact with the microwave radiation and hence rotate. On the other hand, the not so polar bonds (C-C bonds) do not

interact with the microwave radiation and hence remain stationary. Thus, the rotation of the entire molecule does not take place. In other words, the microwave is absorbed by the polar groups present (e.g. –OH groups attached to CMS molecule) which then behave as if they were anchored to an immobile raft and its immobile localized rotations will occur in the microwave region. Consequently, the severing of the rotating polar bonds occurs, which leads to the formation of free radical sites.

Furthermore, the microwave energy absorbed by the water molecules is quickly transferred to the acrylamide molecules, causing dielectric heating which results in severing of their double bond, thus producing another set of free radicals.

The free radicals thus generated by these effects then recombine with each other through initiation, propagation and termination steps to produce the graft copolymer. The proposed mechanism is shown in Figure 5.4.

A recent report [51] related to process optimization describes a new series of six graft copolymers by microwave-initiated method. For the first four graft copolymers (I-IV) (Table 5.1), the exposure time was varied with the concentration of acrylamide and CMS fixed. For the second set of three graft copolymers (III, V-VI), only the acrylamide concentration was varied keeping the other parameters as optimized in the first set.

5.2.2.2.2 Effect of Exposure Time

It is obvious from Table 5.1 that with the increase in exposure time (1– 4 minutes), the percentage grafting increased up to 3 minutes (which is optimized) after which it decreased. This may be because of the fact that, beyond an exposure time of 3 minutes, the prolonged exposure to microwave irradiation may have degraded the polysaccharide backbone. Hence, the percentage grafting and intrinsic viscosity decreased.

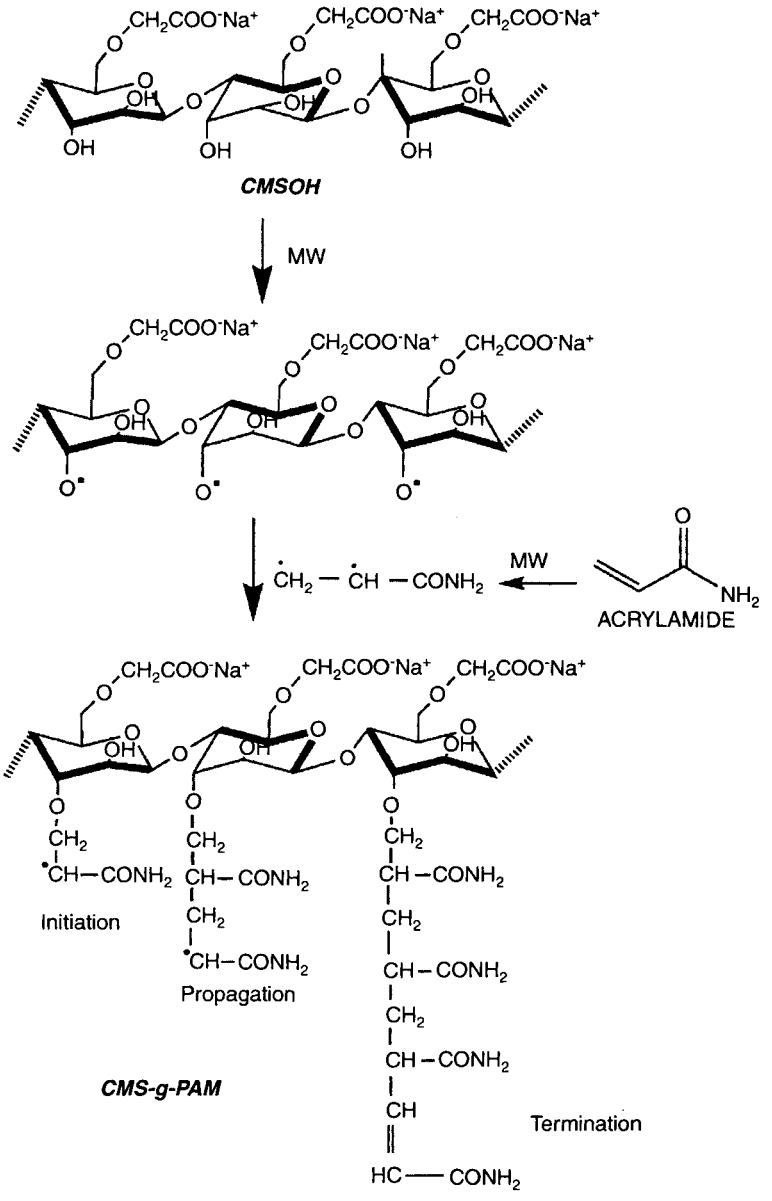
5.2.2.2.3 Effect of Monomer Concentration

The grafting ratio increased upon increasing the monomer concentration from 0.07 moles to 0.14 moles. This increase in percentage grafting may be because of the availability of extra monomer for grafting. But percentage grafting decreased with an increase in the monomer concentration beyond 0.14 moles, which may be due to more homopolymer formation through competing side reaction.

5.2.2.2.4 Interpretation for Using Hydroquinone as an Inhibitor

Inhibitors react with chain radicals to terminate chain propagation and the resulting hydroquinone (HQ) radical is stable and cannot initiate further polymerization. The stability of HQ radical is because of the delocalization of electron charge density throughout the aromatic structure. Hence, the addition of hydroquinone quenches the grafting reaction. This inhibitor action of hydroquinone is a strong support of the free radical mechanism [49, 51].

$$CMSO^{*} + HQ \rightarrow CMSOH + HQ^{*}$$



Formation of homopolymer (competing side reaction)

$$\text{CMSOH} + \text{M} \xrightarrow{\text{MW}} \text{CMSO}^* + \text{M}^*$$

$$\text{M}^* + \text{M} \longrightarrow \text{MM}^*$$

$$\text{M}_{n-1}^* + \text{M} \longrightarrow \text{M}_n^*$$

$$\text{M}_n^* + \text{CMSOH} \longrightarrow \text{CMSO}^* + \text{M}_n\text{H (Homopolymer)}$$

MW - Microwave irradiation
CMSOH - Carboxymethylstarch
M - Monomer (Acrylamide)

Figure 5.4 Mechanism of microwave initiated grafting of PAM chains on CMS backbone.

5.2.2.3 *Purification of the Graft Copolymer by the Solvent Extraction Method*

Any occluded homopolymer formed by competing homopolymer formation reaction should be removed from the grafted polysacharide synthesized by solvent extraction in a suitable solvent where only the homopolymer is highly soluble. In the case of polyacrylamide grafted polysaccharides, a mixture of formamide and acetic acid (1:1 by volume) is used [48, 49, 51].

5.3 Characterization of Grafted Polysaccharides

5.3.1 Intrinsic Viscosity

Viscosity measurements of the polymer solutions are carried out with an Ubbelodhe viscometer. The viscosities are measured in dilute aqueous solution in neutral pH. The time of flow for solutions is measured at four different concentrations (i.e. 0.1%, 0.05%, 0.025% and 0.0125%). From the time of flow of polymer solutions (t) and that of the solvent (t_0, for distilled water), relative viscosity(η_{rel}), specific viscosity (η_{sp}), reduced viscosity (η_{red}) and inherent viscosity(η_{inh}) are obtained by the following relations:

$$\eta_{rel} = t/t_0 \tag{5.1}$$

$$\eta_{sp} = \eta_{rel} - 1 \tag{5.2}$$

$$\eta_{red} = \eta_{sp}/C \tag{5.3}$$

$$\eta_{inh} = (\ln \eta_{rel})/C \tag{5.4}$$

where 'C' is the concentration of the polymer solution.

The intrinsic viscosity is obtained from the point of intersection after the extrapolation of two plots, i.e. 'η_{sp}/C versus C' and '$\ln \eta_{rel}/C$ versus C', to zero concentration [55], as shown in the case of the best grade of polyacrylamide grafted carboxymethylstarch, i.e. CMS-g-PAM V(M) (Figure 5.5).

Intrinsic viscosity [η] of a polymer solution is related to its viscosity average molecular weight by the Mark-Houwink-Sakurada relationship:

$$[\eta] = K.M^a$$

where 'M' represents the viscosity average molecular weight of the polymer, 'K' and 'a' are constants depending on the stiffness of the polymer chain, nature of solvent and temperature.

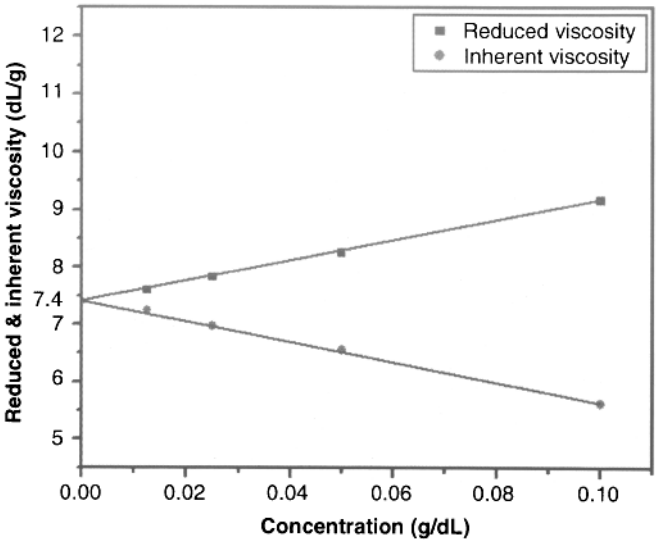


Figure 5.5 Intrinsic viscosity plot for CMS-g-PAM V (M).

In the case of grafted polysaccharides, the grafted polymer chain leads to higher molecular weight, which consequently leads to higher intrinsic viscosity. Thus, an increase in the intrinsic viscosity of the polymer solution with an increase in percentage grafting is a reliable confirmation of the grafting reaction. Furthermore, this increase in intrinsic viscosity with grafting also explains the application of grafted polysaccharides as viscosifier.

The correlation between percentage grafting and intrinsic viscosity in case of polyacrylamide grafted carboxymethylstarch (CMS-g-PAM) is evident in Figure 5.6.

5.3.2 Elemental Analysis

Elemental Analysis (C, H, N, O & S) helps us understand the actual extent of grafting. From the synthesis details (Table 5.1) we get the percentage grafting. Now from percentage grafting and from the known elemental composition of the polysaccharide and that of the graft polymer, we can calculate the theoretical elemental composition, which we can simply compare with that actually obtained from the elemental analyzer.

Furthermore, since most polysaccharide doesn't contain nitrogen, and if the grafted chain is polyacrylamide, then we can confirm grafting simply by observing the presence of nitrogen in the grafted product (and its absence in the polysaccharide) as reported in the case of GG-g-PAM (Table 5.2) [49].

If the grafted chain is polyacrylic acid or polymethyl methacrylate, then we look for an increase in the percentage of oxygen as proof of grafting.

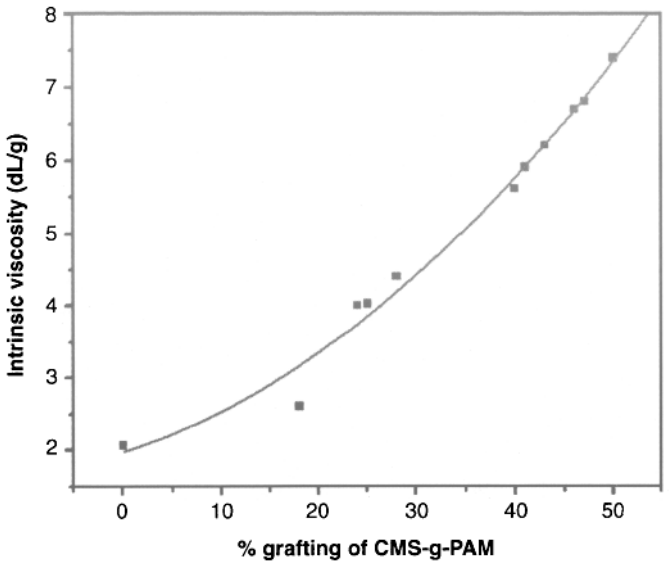


Figure 5.6 Percentage grafting vs intrinsic viscosity for CMS-g-PAM.

Table 5.2 Elemental analysis of guar gum and various grades of polyacrylamide grafted guar (GG-g-PAM)(reprinted from [49]).

Material	%Grafting	Elemental Composition (%)			
		C	H	N	O
Guar Gum	0	52.67	8.35	0.00	38.98
GG-g-PAM 1	32	52.01	7.93	5.30	34.22
GG-g-PAM 2	58	51.86	7.80	7.23	32.66
GG-g-PAM 3	23	52.15	8.03	3.68	35.55
Acrylamide	–	50.80	7.00	19.70	22.50

5.3.3 FTIR Spectroscopy

The FTIR spectroscopy of grafted polysaccharides reveals the presence of specific peaks associated with the vibration of a functional group present in the graft polymer chain but not in the polysaccharide. The presence of these specific peaks confirms the grafting reaction.

The FTIR spectra of starch, carboxymethylstarch (CMS), conventionally and 'microwave initiated' synthesized CMS-g-PAM, i.e. CMS-g-PAM(C) and CMS-g-PAM (M) has been tabulated in Table 5.3.

Table 5.3 FTIR spectra of starch, CMS (best grade), PAM & best grades of conventionally and 'microwave initiated' synthesized CMS-g-PAM.

Polymer	υ OH (cm^{-1})	υ CH (cm^{-1})	υ C-O-C (cm^{-1})	υ COO^- (cm^{-1})	υ CN (cm^{-1})	υ CH_2 Twisting (cm^{-1})	υ CH_2 Scissoring (cm^{-1})	υ C = O (Amide – I) (cm^{-1})	υ NH (Amide – II) (cm^{-1})
Starch	3328	2901	1089 1008	–	–	–	–	–	–
CMS	3319	2935	1080, 1016	1660 1417	–	–	–	–	–
PAM	–	2930	–		1398	1305	1450	1680	1659
CMS-g-PAM IV (C)	3420	2934	1078, 1014	1600 1411	**1400**	**1303**	**1442**	**1672**	**1650**
CMS-g-PAM V (M)	3422	2928	1084, 1010	1605	**1390**	**1300**	**1440**	**1670**	**1646**

The FTIR spectrum of polysaccharide, modified polysaccharide and grafted polysaccharide are explained as below:

In the FTIR spectra of starch, it is observed that a broad peak at 3328 cm^{-1} is due to the stretching vibrations of O-H, and a small peak at 2901 cm^{-1} is attributed to the C-H stretching vibrations. The bands at 1089 cm^{-1} and 1008 cm^{-1} are assigned to C-O-C stretching vibrations.

In the FTIR spectra of CMS 4, it is obvious that apart from the peaks present in starch, there are two additional strong peaks at 1660 cm^{-1} and 1417 cm^{-1} that are attributed to the COO^- groups, which is a clear indication that carboxymethylation does take place.

In the polyacrylamide (PAM) spectra, the strong absorption band at 3430 cm^{-1} is attributed to the N-H stretching vibrations of the primary amide. The two bands at 1680 cm^{-1} and 1659 cm^{-1} are observed due to amide – I ($\upsilon_{C=O}$) and amide –II (υ_{NH}), respectively. The bands at 2930 cm^{-1} and 1398 cm^{-1} are observed and attributed to the C-H and C-N stretching vibrations respectively. Two other bands at 1450 cm^{-1} and 1305 cm^{-1} are attributed to CH_2 scissoring and CH_2 twisting respectively.

Few differences have been observed in the spectra of grafted CMS's i.e. CMS-g-PAM IV (C) and CMS-g-PAM V (M). O-H stretching band of hydroxyl group of CMS and N-H stretching band of amide group of PAM overlap with each other and lead to a broad band, which appears at 3422 cm^{-1} for CMS-g-PAM V (M) [3420 cm^{-1} for CMS-g-PAM IV (C)]. The appearance of two sharp peaks at 1670 cm^{-1} and 1646 cm^{-1} for CMS-g-PAM V (M) [1672 cm^{-1} and 1650 cm^{-1} for CMS-g-PAM IV (C)] are attributed to amide – I ($\upsilon_{C=O}$) and amide –II (υ_{NH}), respectively. Further, there is one more additional band present in both types of grafted products [1390 cm^{-1} for CMS-g-PAM V (M) and 1400 cm^{-1} for CMS-g-PAM IV (C)] which is assigned to the C-N stretching bands. CH_2 scissoring and CH_2 twisting bands appear at 1440 cm^{-1} and 1300 cm^{-1} respectively for CMS-g-PAM V (M) [1442 cm^{-1} and 1303 cm^{-1} for CMS-g-PAM IV (C)]. Thus, the presence of these additional bands in case of grafted polymers compared to CMS confirms the successful grafting of PAM chains onto the backbone of CMS.

5.3.4 Scanning Electron Microscopy (SEM) Analysis

The grafting of polysaccharides results in a profound change in morphology. The morphology of grafted polysaccharides are generally 'fibular', i.e. consisting of fiber- like structures.

The morphology of carboxymethylstarch (CMS), polyacrylamide(PAM) and the best grades of polyacrylamide grafted carboxymethylstarch (conventionally synthesized and microwave initiated) is shown in Figure 5.7.

As shown in Figure 5.7, carboxymethylstarch (CMS) has a granular morphology. But as polyacrylamide was grafted onto the backbone of carboxymethylstarch (CMS), the morphology changed to fibular. This happened in both 'conventional' (i.e. using ceric ammonium nitrate i.e. CAN as free radical initiator) as well as 'microwave initiated' (i.e. using microwave radiation alone to create free radicals onto the CMS backbone) synthesis of the grafted polysaccharide.

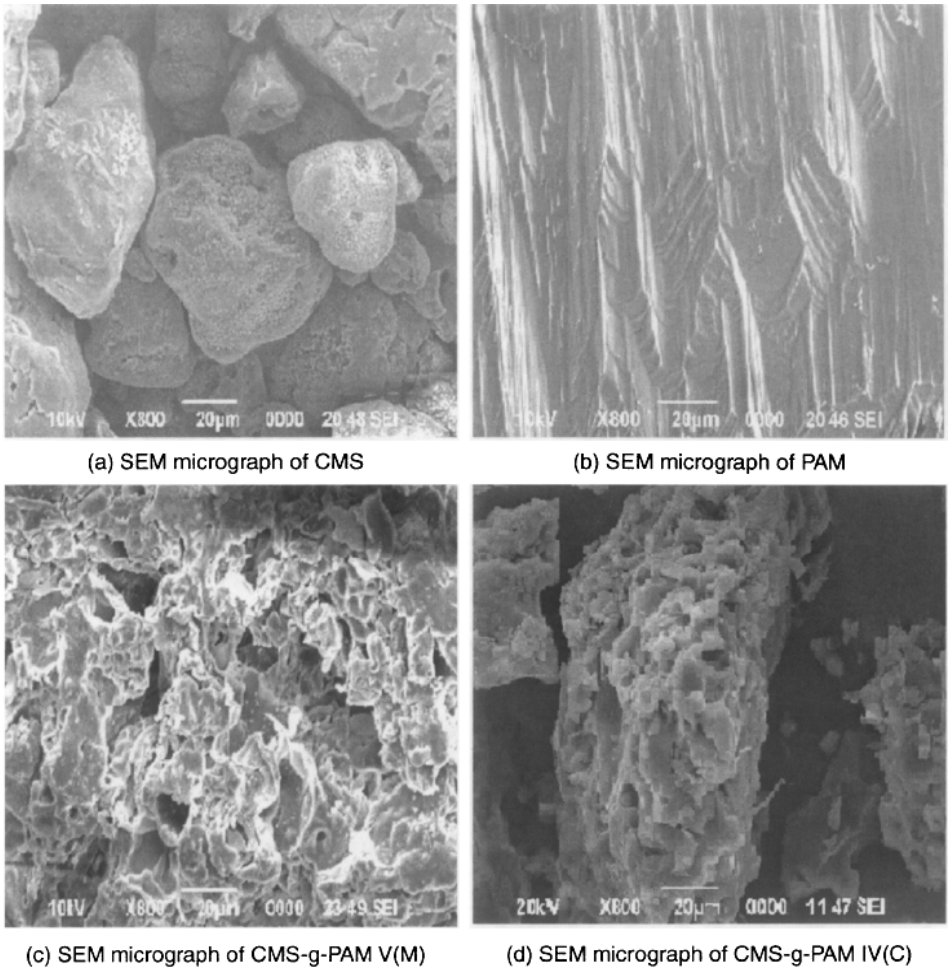


Figure 5.7 SEM Morphology of (a) Carboxymethylstarch (b) Polyacrylamide (c) Polyacrylamide grafted carboxymethylstarch ('microwave initiated') (d) Polyacrylamide grafted carboxymethylstarch (conventional) (reprinted from [51]).

5.3.5 Thermo Gravimetric Analysis (TGA)

The TGA analysis demonstrates the additional 'weight loss' in addition to all the zones of weight loss corresponding to the core polysaccharide. The additional weight loss can be explained by the corresponding grafted polymer chain.

The TGA analysis of starch and of the optimized grades of various synthesized polymeric materials is shown in Figure 5.8a (starch), Figure 5.8b (CMS) and Figure 5.8c (CMS-g-PAM V [M]).

The TGA of starch (Figure 5.8a) has two zones of weight loss. The weight loss at ~100°C is due to the elimination of moisture. The 2nd zone of weight loss at ~ 300°C is due to the degradation of the backbone of the polymer.

The TGA of CMS 4 (Figure 5.8b) has another (3rd) zone of weight loss (apart from the two zones of weight loss as in the case of starch) at ~ 580°C, which is due to the degradation of the incorporated carboxymethyl groups.

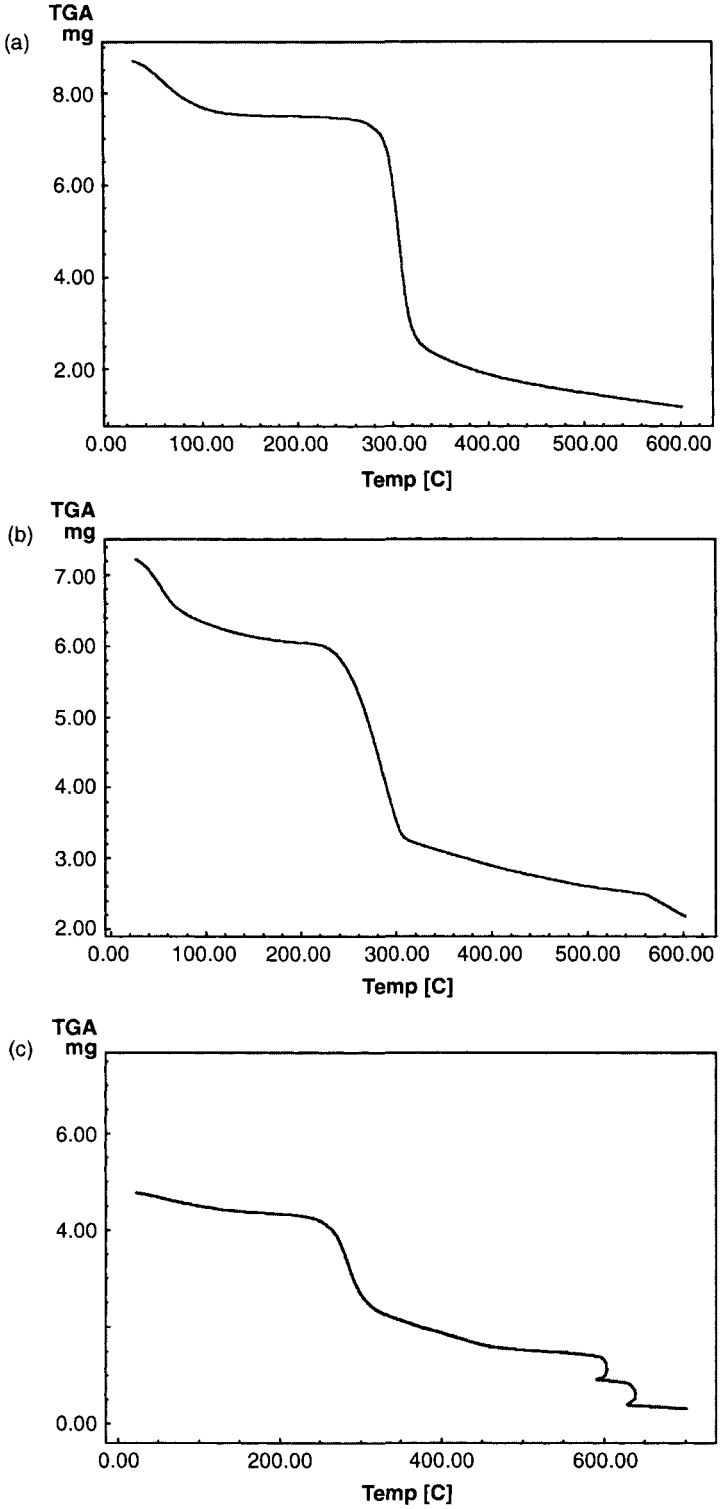


Figure 5.8 (a) TGA of starch (b) TGA of CMS (C) TGA of CMS-g-PAM V (M).

The TGA of CMS-g-PAM V (M) (Figure 5.8c) has another (4^{th}) zone of weight loss (apart from the three zones as described in the case of starch and CMS). This 4^{th} zone of weight loss is at ~ 610°C, which is due to the degradation of the incorporated PAM chains.

5.4 Application of Grafted Polysaccharides

5.4.1 Application as Viscosifier

As previously discussed, the intrinsic viscosity of a polymer solution is predicted by the Mark-Houwink-Sakurada relationship:

$$[\eta] = K.M^a$$

where 'M' represents the viscosity average molecular weight of the polymer, 'K' and 'a' are constants depending on the stiffness of the polymer chain, nature of solvent and temperature.

Thus, the higher the molecular weight, the higher the intrinsic viscosity of the polymer in solution will be.

Due to the presence of grafted chains, graft copolymers can have high molecular weights. Hence, from the Mark-Houwink-Sakurada relationship it is obvious that graft copolymers have high intrinsic viscosity. Thus, although the viscosity of polysaccharide solution is limited by its molecular weight, we can have grafted polysaccharides with much higher molecular weight and hence yielding highly viscous solutions. Such grafted polysaccharides are good candidates for application as viscosifier.

Polyacrylamide grafted polysaccharides are generally nontoxic. Again, as they are modified polysaccharides, they are less likely to be metabolized by our digestive system. Thus, polyacrylamide grafted polysaccharides hold good promise as viscosifier in food preparations (e.g. soft drinks, soups, etc.), imparting the right amount of viscosity without contributing calories.

5.4.1.1 Application of CMS-g-PAM as Viscosifier

The rheological investigations were conducted using a Controlled-Stress TA Instruments AR-1000 Advanced Rheometer. The temperature of the system was maintained at 30 °C throughout the experiments.

The aqueous solutions of polymer samples were prepared by adding the required quantity of the polymers slowly to distilled water to avoid lumping, and with continuous stirring by a magnetic stirrer at a temperature of 60 °C for 4 hours. For CMS and CMS-g-PAM (M), 0.5 wt% solutions were prepared. The rheological characteristics are shown in Figure 5.9a and Figure 5.9b.

In the steady state shear flow test, two main relationships of double logarithmic scales illustrate the rheological fingerprints of the sample under study. These are the flow behavior curve (Figure 5.9a), showing the relationship

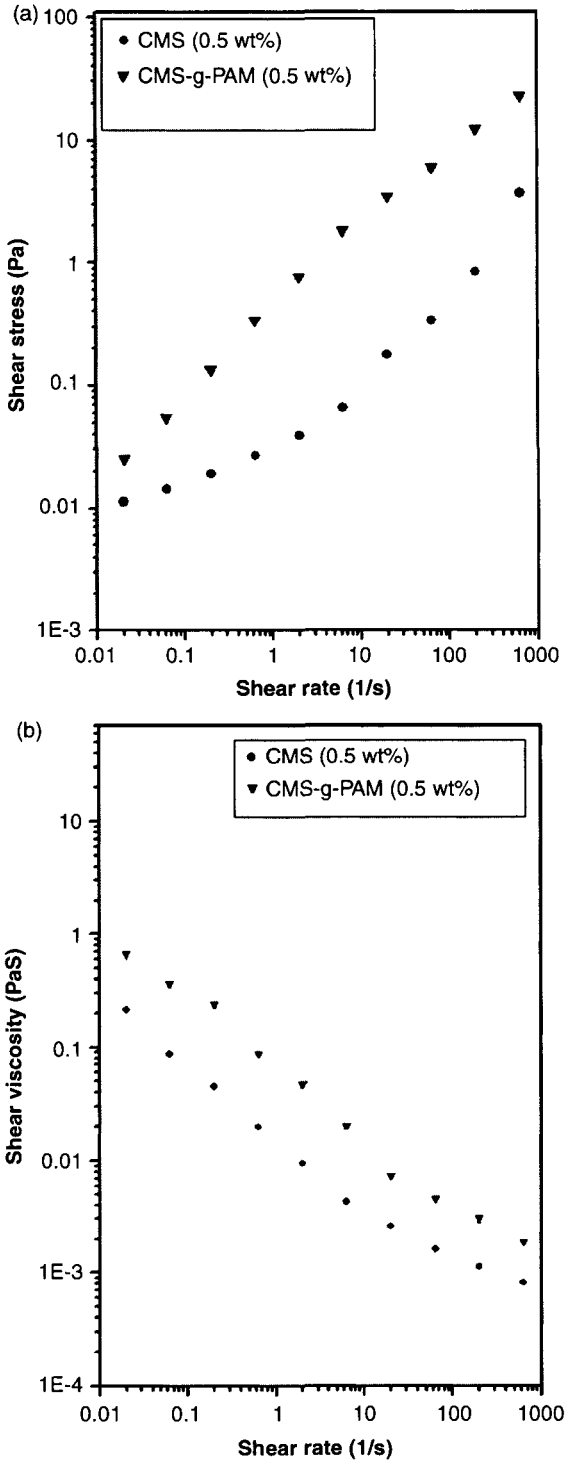


Figure 5.9 (a) Flow behavior curve (b) Viscosity behavior curve.

between shear stress τ and shear rate γ and the viscosity curve (Figure 5.9b) indicating the log-log plots of shear viscosity versus shear rate of aqueous solutions of the polysaccharide (CMS) and its graft copolymer. All the measurements are based on the assigned shear in CR (controlled rate) mode ranging from 0.028 to 2800 s^{-1}. All these polymer solutions show a strong pseudoplastic behavior.

When the polymers were dissolved in solvent, the solvent attached itself to the polymers through hydrogen bonding. The shear rate of the graft copolymer (CMS-g-PAM) is more than the corresponding polysaccharides. This is explained on the basis of the presence of longer PAM chains in the graft copolymer.

The viscosity curve (Figure 5.9b) illustrates the variation of viscosity of polysaccharide and modified polysaccharides (CMS-g-PAM) with shear rate of the aqueous solution of the polymers. The viscosity decreases with the increase in shear rate. This marked shear thinning behavior of polysaccharide solutions may be explained by the conformational states of polymer molecules. At low shear rate or rest, the polysaccharide molecules are stabilized by strong intermolecular hydrogen bonding. On increasing the shear rate the extent of aggregation is reduced, and the viscosity decreases. Figure 5.9b shows the comparison of viscosity of the aqueous solutions of the polysaccharide and its graft copolymer. Both the polymers show shear thinning non-Newtonian behavior. In this case, the viscosity of graft copolymer is more than the base polysaccharide. This is because of the presence of longer PAM chains onto the backbone of CMS, and as a result there will be an increase in hydrodynamic volume resulting in higher viscosity.

5.4.2 Application as Flocculant for Water Treatment

The world's population is increasing, while the availability of potable water is decreasing. Water is essential for the survival of human beings, not to mention modern industry. This necessitates the recycling of municipal wastewater and industrial effluents on a massive scale. To meet the requirements of potable, industrial and agricultural water, the wastewater has to be treated, particularly the municipal sewage, sludges & slimes and industrial effluents. These effluents are highly undesirable and unsafe to use.

Wastewater contains solid particles with a wide variety of shapes, sizes, densities, etc. Specific properties of these particles affect their behavior in liquid phases, and thus their removal capabilities. Many chemical and microbiological contaminants found in wastewater are adsorbed by, or incorporated into, these solid particles. Thus, essential for purification and recycling of both wastewater and industrial effluents, is the removal of these solid particles. Flocculation is a technique where polymers are involved in a solid-liquid separation by an aggregation process of colloidal particles [56]. Both synthetic and natural polymers have been utilized for flocculation.

The materials being used as flocculating agents can be broadly divided into two categories, inorganic and organic. The organic flocculants include

polymers, which are further classified into natural and synthetic. The synthetic polymers may be nonionic, cationic or anionic, while most of the natural polymers are nonionic except in a few cases. Grafting of synthetic polymers onto the backbone of natural polymers (e.g. polysaccharides) constitutes the most advanced class of flocculants.

In the application as flocculant, natural and synthetic polymers have their own advantages and disadvantages. Although natural polymers (i.e. polysaccharides) form stable floc, they are required to be added in high dosages. On the other hand, although synthetic polymeric flocculants are required to be added in minute dosage, the flocs formed in this case are fragile. The grafting of synthetic polymer onto natural polysaccharides yields 'hybrid' flocculants with the advantages of both.

5.4.2.1 Bridging Mechanism – The Theory behind Nonionic Flocculants

Ruehrwein and Ward first proposed the basic principle of bridging flocculation in 1952 [57]. They presented a model where a single polymer chain was bridging two or more colloidal particles. Although the basics of this model have been subsequently refined, the major points are unchanged, i.e. the loops and tails of the adsorbed polymer structure on one particle protrude into solution and get attached to a second particle. Smellie and La Mer [58] postulated that the surface coverage of adsorbed polymer is a fundamental parameter controlling the probability of bridging. Subsequently, Healy and La Mer [59] introduced the concept of 'half surface coverage' as being the optimum condition for flocculation to occur.

When long chain polymers in small dosages are added to a colloidal suspension, they get adsorbed onto two or more particle surfaces thereby forming a bridge between them. There should be sufficient unoccupied space on the particle surface so as to form polymer bridging. This phenomenon is observed up to a particular dosage of polymer beyond which flocculation diminishes, the process being known as 'steric stabilization'. Hence, at lower dosages of polymer, there is no adequate bridging occurring between the particles. Similarly at higher dosages of polymer, there is insufficient particle surface for attachment of the polymer segments, leading to destabilization. Evidently, there is an optimal dosage of the flocculant (polymer) for effective flocculation. Beyond this dosage, destabilization of the floc (in other words, restabilization of the colloidal solution) takes place. The effective dosage of flocculant and the flocculation efficacy of the flocculant are determined through 'jar test' procedure.

5.4.2.2 Singh's Easy Approachability Model

Singh's easy approachability model [60–65] explains the superiority of grafted polysaccharides over other types of flocculants. This model states that the dangling branches of polyacrylamide or cationic moiety have easy approachability when they are grafted onto the rigid polysaccharide backbone.

Furthermore, the more there is of the branched base of the polysaccharide, the greater the opportunity the grafted (onto polysaccharide backbone) polyacrylamide/cationic moiety chain will have to form an aggregate of the contaminants. Thus, if the grafted moiety is loaded onto the most hyperbranched polysaccharides, it will have easier accessibility to form aggregates of the contaminants, providing the best flocculation characteristics.

5.4.2.3 *Flocculation Characteristics of Polyacrylamide Grafted Polysaccharides*

Flocculation efficacy and optimum dosage of flocculants are evaluated in 'jar test' apparatus, which is expected to simulate the behavior of the flocculant when applied on a large scale, e.g. in a wastewater treatment plant. The apparatus consists of a series of identical glass jars, which can be identically stirred by overhead mechanical stirrers. The jars contain the wastewater/colloidal suspension with progressively varying dosage of the flocculant. A typical 'jar test' protocol consists of stirring the jars at 150 rpm for half minutes, followed by stirring at 60 rpm for 5 minutes and then settling for 15 minutes, and then collecting the supernatant liquid and measuring its turbidity. The result is expressed graphically as turbidity vs. flocculant dosage. The flocculation characteristics of microwave initiated synthesized polyacrylamide grafted carboxymethylstarch (CMS-g-PAM) in 0.25% coal fine suspension is shown in Figure 5.10. It is evident that the higher the percentage of grafting, the higher the flocculation efficacy. Thus, CMS-g-PAM I(M) which has a percentage grafting of just 25% (Table 5.1) showed the least flocculation efficacy, while CMS-g-PAM V(M) which has the highest percentage grafting (50%) showed maximum flocculation efficacy.

5.4.3 Application as Matrix for Controlled Drug Release

One major challenge in the field of pharmacokinetics is to sustain the therapeutic level of a drug in body tissue (e.g. blood) as long as possible, during the entire length of treatment.

Traditional delivery systems (TDS) (Figure 5.11a) are characterized by immediate and uncontrolled drug release kinetics. In this case, drug absorption is essentially controlled by the body's ability to assimilate the therapeutic molecule and thus, drug concentration in different body tissues such as the blood, typically undergoes an abrupt increase on administration of the dosage, followed by a similar decrease. As a consequence, the drug concentration might approach the toxic threshold and after some time, fall down below the effective therapeutic level (therapeutic level of a drug is its concentration in the body tissue such as blood, at which it performs its intended function without causing any significant side effects) [66, 67].

The side effects always occur with high doses and are proportional to its concentration in the blood. Evidently, the initial high concentration (just after traditional drug administration) of the drug in the blood leads to unwanted

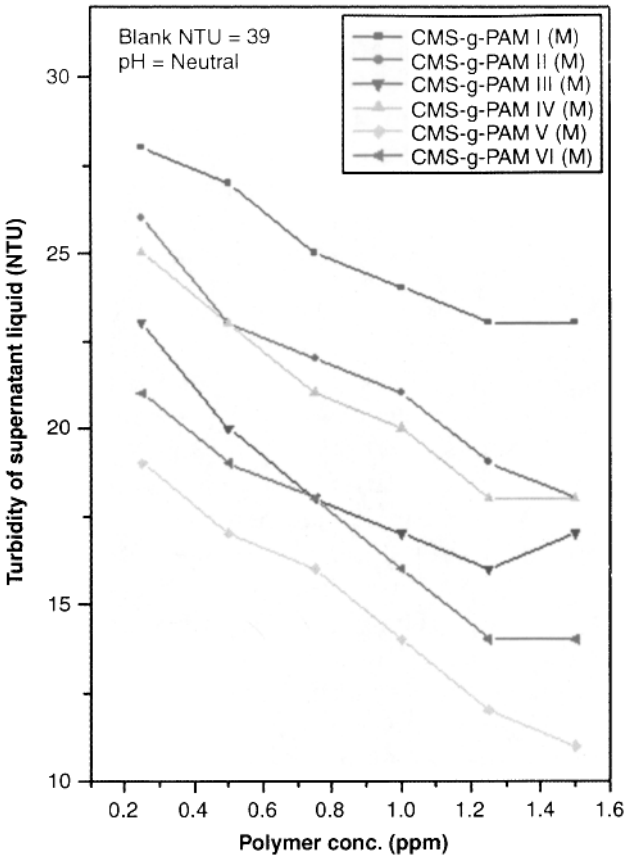


Figure 5.10 Flocculation characteristics of various grades of microwave initiated synthesized CMS-g-PAM, in 0.25% coal suspension.

side effects. Again, the subsequent sub-therapeutic level may lead to gradual development of drug resistance. It is obvious that in TDS, one has to struggle to maintain the therapeutic level of the drug by the repeated and frequent administration of the dosage. This in turn leads to the patient's inconvenience.

On the contrary, the purpose of controlled release systems (CRS) is to maintain drug concentration in the target tissues (e.g. blood) at the therapeutic level as long as possible (Figure 5.11b). Thus, both the high concentrations (peaks) and the subsequent sub-therapeutic levels (troughs) of the drug associated with TDS are avoided. Consequently, this minimizes the side effects and also reduces the chances of drug resistance [66, 67]. Furthermore, the low frequency of dosage leads to the patient's convenience.

The most convenient controlled release system (CRS) consists of the drug enclosed in a customized matrix, which releases the drug at a predetermined rate, to maintain the therapeutic level of the drug in blood plasma or in the area of the intended tissue.

Because of low cost and ease of fabrication, one common way of obtaining the controlled release of a drug is by enclosing it in a hydrophobic matrix (such

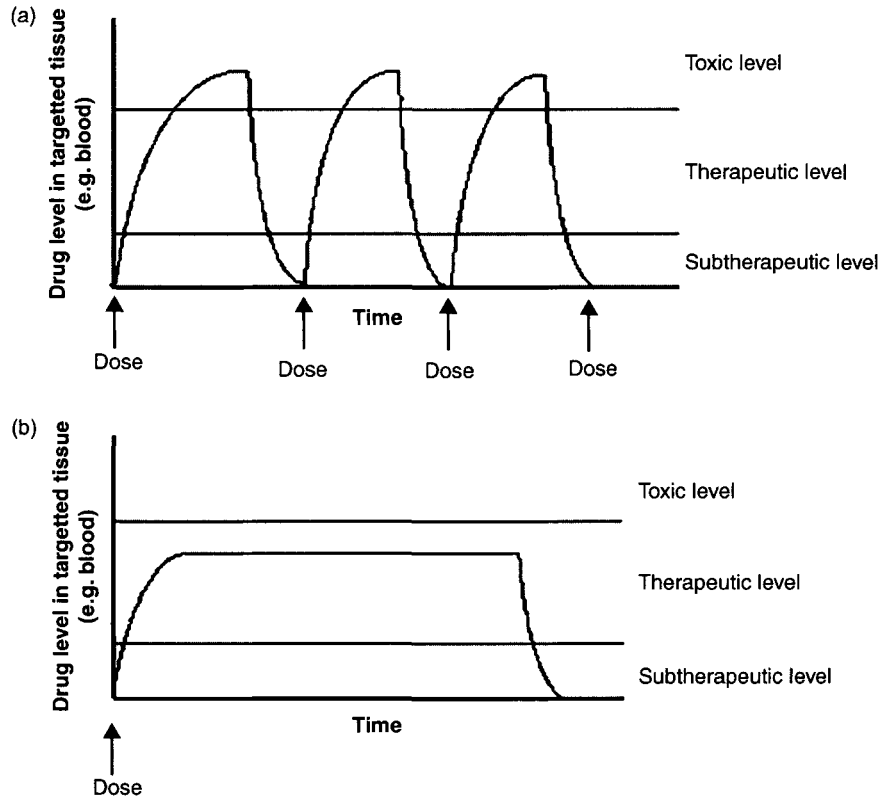


Figure 5.11 Drug level in body tissue (e.g. blood) in case of (a) traditional drug delivery system (TDS) (b) controlled drug delivery system(CDS).

as wax, polyethylene, polypropylene, and ethyl cellulose) or in a hydrophilic matrix (such as carboxymethyl tamarind, hydroxypropylcellulose, hydroxypropylmethylcellulose, methylcellulose, sodium carboxymethylcellulose, alginates and scleroglucan) [68–99].

The application of grafted polysaccharides as matrix for controlled drug release is of current research interest. The higher the percentage grafting, the higher the branching and molecular weight of the grafted polysaccharide will be. The entanglement between the graft chains of neighbouring macromolecules of the grafted polysaccharide makes dissolution difficult. Thus, the higher the percentage grafting, the lower the solubility of the grafted polysaccharide will be. Hence, the erosion rate of any tablet made using the grafted polysaccharide as 'matrix' will be lower i.e. the rate of release of the enclosed drug will be lower. In other words, the higher the percentage grafting of a grafted polysaccharide, the lower (or more sustained) will be the rate of release of the drug enclosed in it. Thus, use of grafted polysaccharide as matrix for controlled drug release has the advantage of programming the rate of drug release at a molecular level, during synthesis, in terms of percentage grafting. A number of recent *'in-vitro'* studies [34, 47, 49] of drug release from grafted

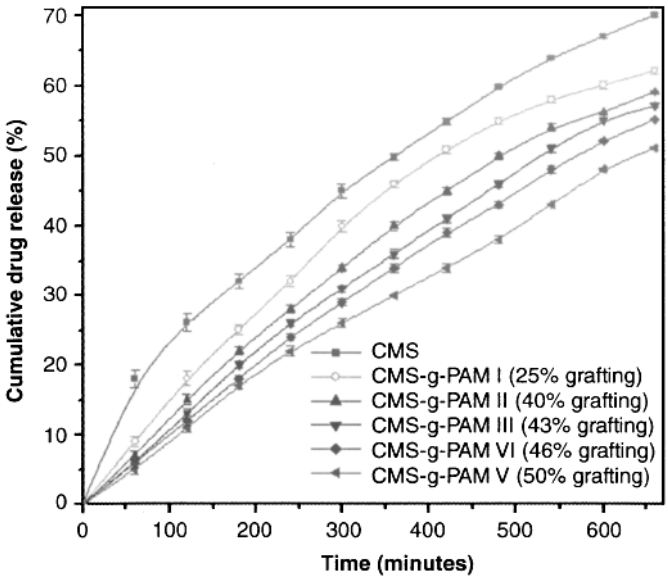


Figure 5.12 Drug release profile in neutral (pH = 7.0) dissolution medium. The results are mean ± S.D. (n = 3). (reprinted from [47]).

polysaccharide matrix has demonstrated this phenomenon. A recent study [47] has even reported an empirical relationship between 'percentage grafting' and rate of drug release from a grafted polysaccharide (CMS-g-PAM), valid for all types of dissolution medium (acidic, neutral and alkaline).

The *in vitro* drug release profile (USP drug dissolution method, paddle type) of 5-amino salicylic acid from various grades of CMS-g-PAM matrix, in neutral dissolution medium is shown in Figure 5.12 [47].

5.5 Conclusion

Polysaccharides are renewable and they are also the most abundant organic compounds on this planet. The properties of polysaccharides can be customized according to our needs, through chemical modification, the most effective of which is grafting with a synthetic polymer such as polyacrylamide (PAM). There are various techniques of grafting available, however, the most convenient and efficient techniques are the microwave-based methods. There are diverse uses possible for grafted polysaccharides, the most significant are as flocculant for wastewater treatment, as matrix for controlled drug release and rheological applications.

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6

Chitosan: The Most Valuable Derivative of Chitin

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Abstract

Chitosan or P-(1,4)-2-amino-2-deoxy-D-glucose, is a hydrophilic biopolymer obtained industrially by hydrolyzing the amino acetyl groups of chitin, which is the main component of shells of crab, shrimp and krill, by an alkaline treatment. Chitosan derived from chitin, possesses a unique cationic nature relative to other neutral or negatively charged polysaccharides. The amino group, NH_2, in chitosan can be protonated to NH_3^+ in an acid environment, resulting in anti-fungal or antimicrobial activities, since cations can bind with anionic sites in protein. Furthermore, chitosan is a nontoxic natural polysaccharide and is compatible with living tissue. These distinguishing features make chitosan widely applicable in healing, artificial skin, food preservation, cosmetics, and wastewater treatment. However, the hydrophilic character of chitosan and consequently its poor mechanical properties in the presence of water and humid environments, limits its application. Blending chitosan with other polymers such as polylactic acid, polycaprolactone, etc., or developing chitosan-layered silicate nanocomposites by inserting chitosan chains into an interlayer of silicate can improve its mechanical, thermal, and biological properties. The objective of this chapter is to briefly describe the characterization, chemical modification, de-polymerization and applications of chitin and chitosan. An emphasis will be placed on recent studies and research regarding high value adding applications of these materials for wastewater treatment, packaging, and in different branches of the biomedical field.

Keywords: Chitosan, modification of chitosan, biomedical applications

6.1 Introduction

Use of natural biopolymers for diversified applications in life sciences has several advantages, such as their availability from replenishable agricultural or marine food resources, biocompatibility, and biodegradability, therefore leading to ecological safety and the possibility of preparing a variety of chemically or enzymatically modified derivatives for specific end uses. Polysaccharides, as a class of natural macromolecules, have the tendency to be extremely bioactive, and are generally derived from agricultural feedstock or crustacean shell

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (129–166)

wastes. Cellulose, starch, pectin, etc., are the biopolymers derived from the former while chitin and chitosan are obtained from the latter. In terms of availability, chitin is next to cellulose, available to the extent of over 10 gigatons annually. The application potential of chitosan, a deacetylated derivative of chitin, is multidimensional, such as in food and nutrition, biotechnology, material science, drugs and pharmaceuticals, agriculture and environmental protection, and recently also in gene therapy. The net cationicity as well as the presence of multiple reactive functional groups in the molecule make chitosan a sought-after biomolecule. The latter offers scope for manipulation for preparing a broad spectrum of derivatives for specific end use applications in diversified areas. The biomedical and therapeutic significance of chitin/chitosan derivatives is a subject of significant concern to many all over the world. This overview will attempt to consolidate some of the recent findings on the biorelated application potential of chitosan and its derivatives.

6.2 Polysachharide

By far the majority of carbohydrate materials in nature occur in the form of polysaccharides. By our definition, polysaccharides include not only those substances composed only of glycosidically linked sugar residues, but also molecules that contain polymeric saccharide structures linked via covalent bonds to amino acids, peptides, proteins, lipids and other structures. Polysaccharides, also called glycans, consist of monosaccharide and their derivatives. If a polysaccharide contains only one kind of monosaccharide molecule, it is known as a homopolysaccharide, or homoglycan, whereas those containing more than one kind of monosaccharide are heteropolysaccharides [1]. The most common constituent of polysaccharides is D-glucose, but D-fructose, D-galactose, L-galactose, D-mannose, L-arabinose, and D-xylose are also frequent. Some monosaccharide derivatives found in polysaccharides include the amino sugars (D-glucosamine and galactosamine) as well as their derivatives (*N*-acetylneuraminic acid and *N*-acetylmuramic acid), and simple sugar acids (glucuronic and iduronic acids). Homopolysaccharides are often named for the sugar unit they contain, so glucose homopolysaccharides are called glucans, while mannose homopolysaccharides are mannans [2]. Polysaccharides differ not only in the nature of their component monosaccharides but also in the length of their chains and in the amount of chain branching that occurs [3]. Although a given sugar residue has only one anomeric carbon and thus can form only one glycosidic linkage with hydroxyl groups on other molecules, each sugar residue carries several hydroxyls, one or more of which may be an acceptor of glycosyl substituents. This ability to form branched structures distinguishes polysaccharides from proteins and nucleic acids, which occur only as linear polymers [4, 5].

The main functions played by polysaccharides in Nature are either storage or structural functions. By far the most common storage polysaccharide in plants is starch, which exists in two forms: α- amylose and amylopectin [6].

Structural polysaccharides exhibit properties that are dramatically different from those of the storage polysaccharides, even though the compositions of these two classes are similar. The structural polysaccharide cellulose is the most abundant natural polymer in the world. Found in the cell walls of nearly all plants, included marine algae, cellulose is one of the principal components, providing physical structure and strength [1].

Chitin is the second most abundant organic compound in nature after cellulose [7]. Chitin is widely distributed in marine invertebrates, insects, fungi, and yeast [8]. However, chitin is not present in higher plants and higher animals. They are important not only because they are an abundant resources, but also for their attracting biological properties and potential in the biomedical field [8].

6.3 Sources of Chitin and Chitosan

Chitin is widely distributed in marine invertebrates (Figure 6.1), insects, fungi, and yeast [9]. However, chitin is not present in higher plants and higher animals. Generally, the shell of selected crustacean was reported by Knorr [10] to consist of 30-40% protein, 30-50% calcium carbonate and calcium phosphate, and 20-30% chitin. Chitin is widely available from a variety of sources among which, the principal source is shellfish waste such as shrimps, crabs, and crawfish [11]. It also exists naturally in a few species of fungi. In terms of its structure, chitin is associated with proteins and, therefore, high in protein content. Chitin fibrils are embedded in a matrix of calcium carbonate and phosphate that also contains protein. The matrix is proteinaceous, where the protein is hardened by a tanning process [12]. It was also demonstrated that

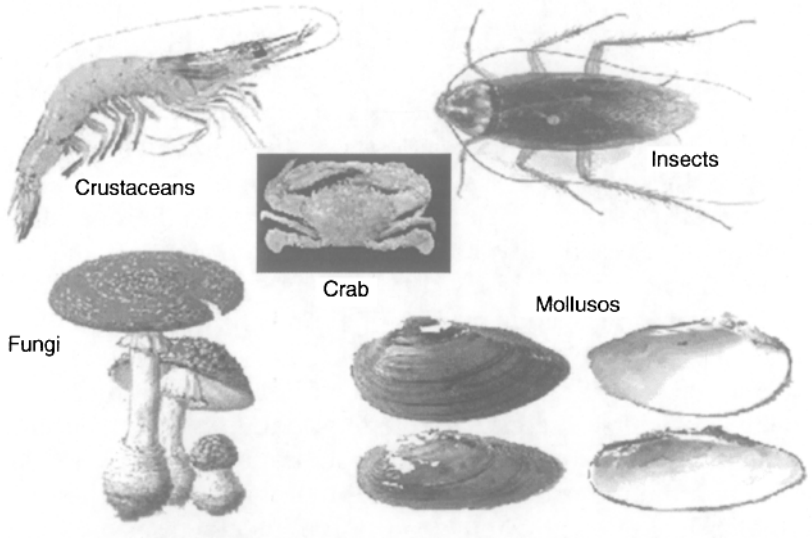


Figure 6.1 Sources of Chitin [1].

chitin represents 14–27% and 13–15% of the dry weight of shrimp and crab processing wastes, respectively [13].

6.4 Composition of Chitin, Chitosan and Cellulose

Chitosan is a modified natural carbohydrate polymer derived from chitin which has been found in a wide range of natural sources such as crustaceans, fungi, insects and some algae [14]. Removal of most of the acetyl groups of chitin by treatment with a strong alkali yields chitosan [15] which is 2-amino-2-deoxy-β-D-glucose. A sharp nomenclature with respect to the degree of *N*-deacetylation has not been defined between chitin and chitosan [12]. In general, chitin with a degree of deacetylation of above 70% is considered as chitosan [16].

Chitosan is insoluble in water but soluble in acidic solvents below pH 6. Organic acids such as acetic, formic and lactic acids are used for dissolving chitosan, and the most commonly used solvent is 1% acetic acid solution. Solubility of chitosan in inorganic acid solvent is quite limited. Chitosan is soluble in 1% hydrochloric acid but insoluble in sulfuric and phosphoric acids. Chitosan solution's stability is poor above pH 7 due to the precipitation or gelation that takes place in the alkali pH range. Chitosan solution forms a poly-ion complex with anionic hydrocolloid to produce a gel.

With regard to its chemical structure (Figure 6.2), chitosan is very much similar to cellulose and chitin. Chitin is made up of a linear chain of acetylglucosamine groups while chitosan is obtained by removing enough acetyl groups (CH_3-CO) for the molecule to be soluble in most diluted acids. This process is called deacetylation. The actual difference between chitin and chitosan is the acetyl content of the polymer. Chitosan having a free amino group is the most useful derivative of chitin [17]. It is also very much similar to cellulose, a plant fiber. As seen in Figure 6.2, the only difference between chitosan and cellulose is the amine (-NH_2) group in the position C-2 of chitosan instead of the hydroxyl (-OH) group found in cellulose. However, unlike plant fiber, chitosan possesses positive ionic charges, which give it the ability to chemically bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and macromolecules [18]. In this respect, chitin and chitosan have attained increasing commercial interest as suitable resource materials due to their excellent properties including biocompatibility, biodegradability, adsorption, and ability to form films, and to chelate metal ions [19].

Chitosan is a non-toxic, biodegradable polymer of high molecular weight that is widely employed in many biomedical fields [20-24]. Like alginate, chitosan has the characteristic of being able to form gels, in addition to possessing viscosity-related properties, complete biodegradability, and even anti-tumor influence [25]. Its bacteriostatic and fungistatic properties are particularly useful for wound treatment. Furthermore, chitosan possesses bioadhesive properties which makes it of interest when bioadhesive sustained release formulation is required [26-27].

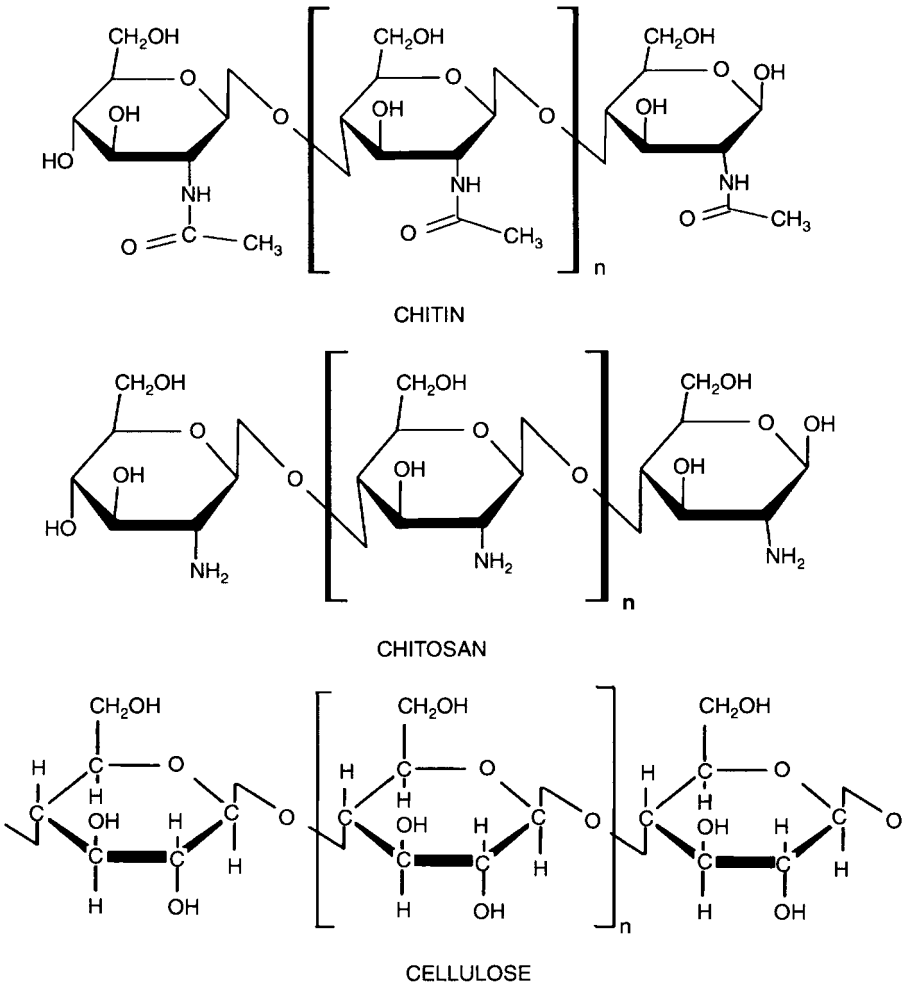


Figure 6.2 The structure of chitin, chitosan and cellulose [1].

Many chitosan derivatives are also biocompatible and non-toxic with living tissues. Recent studies further indicate that chitosan and its derivatives also are novel scaffold materials for tissue engineering and are promising non-viral vectors for gene delivery [28-29]. For bone regeneration, several injectable materials based on chitosan and its derivatives have been used. Chitosan-calcium phosphate (CP) composites appear to have a promising clinical application. Chemically modificated HA (hyaluronic acid)-chitin and chitosan-HA material were reported to be osteoinductive and exhibited rapid degradation and neovascularization *in vivo* [30-31]. Also, chitosan scaffolds are potentially a useful alternative to synthetic cell scaffolds for cartilage tissue engineering [32]. Recently, biomineralized alginate/chitosan microcapsules have been proposed as multifunctional scaffolds and delivery vehicles in tissue regeneration of hard and soft tissues [33].

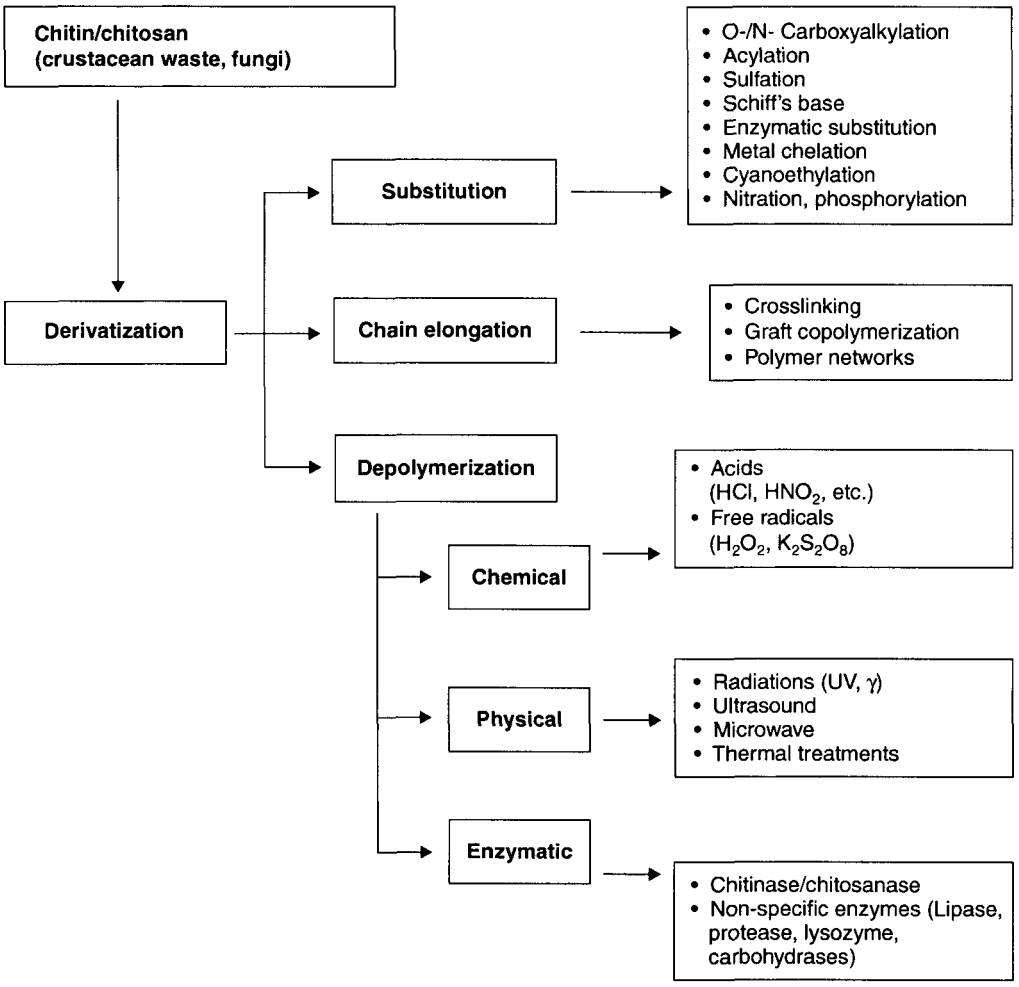
6.5 Chemical Modification of Chitin and Chitosan

The chemical modification of chitin and chitosan is summarized in Table 6.1.

6.6 Chitin – Chemical Modification

Chemical modifications of chitin are generally difficult owing to the lack of solubility because reactions under heterogeneous conditions are accompanied by various problems such as the poor extent of reaction, difficulty in region-selective substitution, structural ambiguity of products and partial degradation due to the severe reaction conditions. Carboxymethyl chitin is one of the most studied chitin derivatives, obtained by adding monochloroacetic acid to chitin previously treated with sodium hydroxide at different

Table 6.1 The chemical modification of chitin and chitosan [1].



concentrations until a neutral viscous milky solution was obtained. The water soluble product was carboxymethyl chitin [34]. For each concentration of alkali used, one set of carboxymethylation degree was allowed to take place [34]. This product activates peritoneal macrophages *in vivo*, suppresses the growth of tumor cells in mice, and stimulates nonspecific host resistance against *Escherichia coli* infections [35]. Chitin can be used in blends with natural or synthetic polymers; it can be crosslinked by epichlorhydrin and glutaraldehyde [36].

The most important chitin derivative is chitosan, obtained by its partial deacetylation under alkaline conditions or by enzymatic hydrolysis in the presence of chitin deacetylase. Because of the semicrystalline morphology of chitin, chitosan obtained by a solid-state reaction has a heterogeneous distribution of acetyl groups along the chain. On the other hand when chitin is treated with concentrated aqueous sodium hydroxide, *N*-deacetylation proceeds smoothly and homogeneously deacetylated samples are obtained [37].

6.7 Chitosan – Chemical Modification

Among the many chemical derivatives of chitosan mentioned in literature [38], one can differentiate the specific reaction involving the $-NH_2$ group at the C-2 position, or the non-specific reactions of –OH groups at C-3 and C-6 positions (especially esterification and etherification). The more common and easier reactions involving the amino group at C-2 position are the quaternization and the reductive amination with aldehydes [39].

6.7.1 O-/N-carboxyalkylation

O-/N-carboxymethylchitosan (CM-chitosan) is one of the most investigated derivatives of chitosan, obtained under controlled reaction conditions with sodium monochloroacetate. This amphoteric polyelectrolyte has attracted considerable interest in a wide range of biomedical applications, such as wound dressings, artificial bone and skin, bacteriostatic agents, and blood anticoagulants, due to its unique chemical, physical, and biological properties, especially its excellent biocompatibility [40-44]. The presence of both carboxyl groups and amino groups in CM-chitosan macromolecules elicits special physicochemical and biophysical properties. It is of interest for use in pharmaceutical applications because of their novel properties, especially for controlled or sustained drug-delivering systems [45]. *N*-carboxymethylation of chitosan is affected through Schiff base formation from the free amino group of chitosan with an aldehyde or keto group and the successive reduction with cyanoborohydride or sodium borohydride [46].

This method results in regioselective carboxymethylation of the amino group, so the product of reaction is a well-defined derivative. Several *N*-carboxyalkylated chitosans were prepared via Schiff base formation from carboxylic acids having aldehyde or keto groups [47-48]. The resulting

carboxyalkylated derivatives find applications as biomedical materials and fungistatic agents [49-51].

O-carboxymethy lchitosan is also used to develop a water-soluble matrix polymer for controlled drug release. OCM-chitosan microspheres containing antibiotic drug pazufloxacin mesilate were prepared by the emulsion method and successively crosslinked with glutaraldehyde [52].

6.7.2 Sulfonation

Chemical modification of the amino and hydroxyl groups of chitosan with sulphate can generate products for pharmaceutical applications. Sulfonation reactions of polysaccharides can give rise to a structural heterogeneity in the polymer chain, but on the other hand some structures that emerge from random distribution can reveal good features for biological functions. Sulphated chitosans that represent the nearest structural analogues of the natural blood anticoagulant heparin, show anticoagulant, antisclerotic, antitumor and antiviral activities [53-56]. Chitosan derivatives having *N*- and/or *O*-sulphate groups either alone or in conjunction with other substituents have been widely examined as potential heparinoids [57]. Chitosan sulphates can be synthesized by sulfation of low molecular weight chitosan (Mw 9000–35,000 Da). When oleum is used as a sulfating agent and dimethylformamide as the medium and it was demonstrated that chitosan sulphates with reduced molecular weight show a regular increase of anti-coagulant activity, such as heparins [58]. Holme *et al.* [59] converted amino groups of chitosan, with low *N*-acetyl content, into anionic centers through *N*-sulfation. They used trimethylamine-sulfur trioxide, which is known to affect selective *N*-sulfation of amino alcohols [60]. Selective *O*-sulfonation of chitosan was performed by Zhang *et al.* [61]. They prepared *N*alkyl- *O*-sulphate chitosan by treating *N*-octyl-chitosan with DMF and chlorosulphonic acid. The thermal stability of such *N*-alkyl-*O*-sulphated chitosan decreased with respect to that of the original chitosan. The introduction of substituents into polysaccharide structures disrupts the crystalline structure of chitosan, especially due to loss of hydrogen bonding. *N*-Alkyl-*O*-sulphate chitosan has an amphiphilic character due to the presence of hydrophobic moieties, alkyl chains, and hydrophilic moieties. Because of this, it has the capacity to form micelles in water and can be used as a potential drug carrier.

6.7.3 Acylation

A variety of acylation reactions of chitosan are possible by using different acylating agents, such as aliphatic carboxylic acid chlorides (hexanoyl, dodecanoyl and tetradecanoyl chlorides), cyclic anhydrides and cyclic esters. The acylation reaction is not regioselective. When *N,O*-acylated chitosans were prepared with acyl chlorides in methanesulfonic acid [62-63], the derivatives of 4-chlorobutyl and decanoyl chlorides showed higher fungidal activities than chitosan [64]. Selectively *N*-acylated chitosan have been obtained by Lee *et al.* [65-66] with butanoic, hexanoic and benzoic anhydride under homogeneous conditions

in the presence of methanol. Such a chemical modification of chitosan was carried out to induce a hydrophobic nature to the hydrophilic chitosan backbone and to prevent particle aggregation. Chitosan nanoparticulate systems for intravenous administration, with an assumption that engineered nanoparticle systems can adsorb overdosed drugs selectively and rapidly, and reduce their free blood concentration to a safe level, were developed [67].

Chitosan can be modified with succinic anhydride in order to obtain a water soluble polymer, Nsuccinylchitosan. The reaction, described by Aiedeh *et al.* [68], was performed under homogeneous conditions in the presence of pyridine and lead to a high degree of chitosan succinylation. The succinylation reaction (illustrated in Figure: 6.3) consists of a condensation reaction between the polysaccharide amine group and the electrophilic carbonyl group of the anhydride. The reaction involves the formation of an amidic bond with the opening of the anhydride ring:

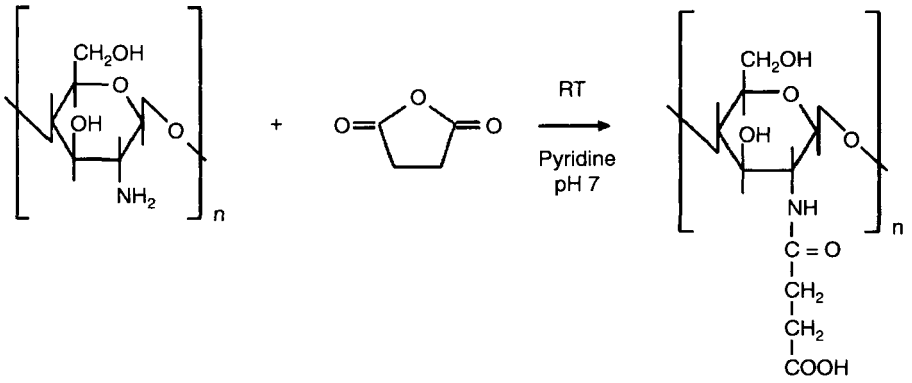


Figure 6.3 Acylation of chitosan [1].

6.7.4 Sugar-Modified Chitosan

The first report on the modification of chitosan with sugars was by Hall and Yalpani [69-70].They synthesized sugar-bound chitosan by reductive *N*-alkylation with $NaCNBH_3$ using either an unmodified disaccharide (Figure 6.4, method A) or a monosaccharide-aldehyde derivative (Figure 6.4, method B). This type of modification has generally been used to introduce cell-specific sugars onto chitosan.

Following this route, Kato *et al.* prepared lactosaminated *N*-succinylchitosan and its fluorescein thiocarbanyl derivative as a liver-specific drug carrier in mice through a sialoglycoprotein receptor [71]. Galactosylated chitosan prepared from lactobionic acid (LA) and chitosan with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) showed promise as a synthetic extracellular matrix for hepatocyte attachment [72]. Yang *et al.* [73-74] prepared the chitosan derivatives through the reductive *N*-alkylation of chitosan as described by Sashiwa and Shigemasa

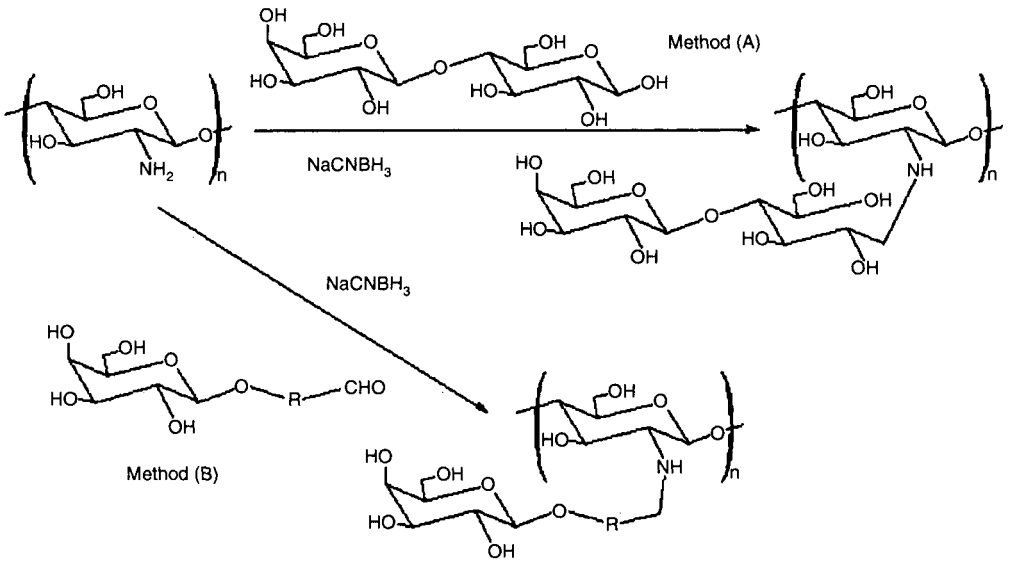


Figure 6.4 Sugar-modification of chitosan [1].

[75] with various mono- and disaccharides. *N*Alkylated chitosan effectively showed solubility at neutral and basic pH regions. Moreover, some derivatives substituted with disaccharides including lactose, maltose and cellobiose showed the solubility at all pH ranges. These derivatives will overcome the application limit of chitosan represented by its reduced solubility.

6.8 Depolymerization of Chitin and Chitosan

The very high molecular weight, and therefore very high viscosity of chitosan, precluded its use in several biological applications. For some specific applications, more than the chitosan, its degradation products, such as LMWC, COs and monomers, were found to be much more useful. A variety of degradation methods - chemical, physical and enzymatic - are being worked out to generate these degradation products (Figure 6.5). Both chitin and chitosan oligomers possess additional functional properties such as antitumor activity [76-78], immuno-enhancing effects, and enhancing protective effects against infection with some pathogens in mice [79], antifungal [80-81] and antimicrobial [82] activities. Additionally, they have lower viscosity, low molecular weights and short chain lengths and are soluble in neutral aqueous medium. Subsequently, they seem to be readily absorbed *in vivo*.

6.8.1 Chemical Methods

Acid degradative methods are not specific, the hydrolysis randomly generates a large amount of monomers, and later on the removal of acid poses problems and is not economical. Chemical treatment using strong acids (viz.

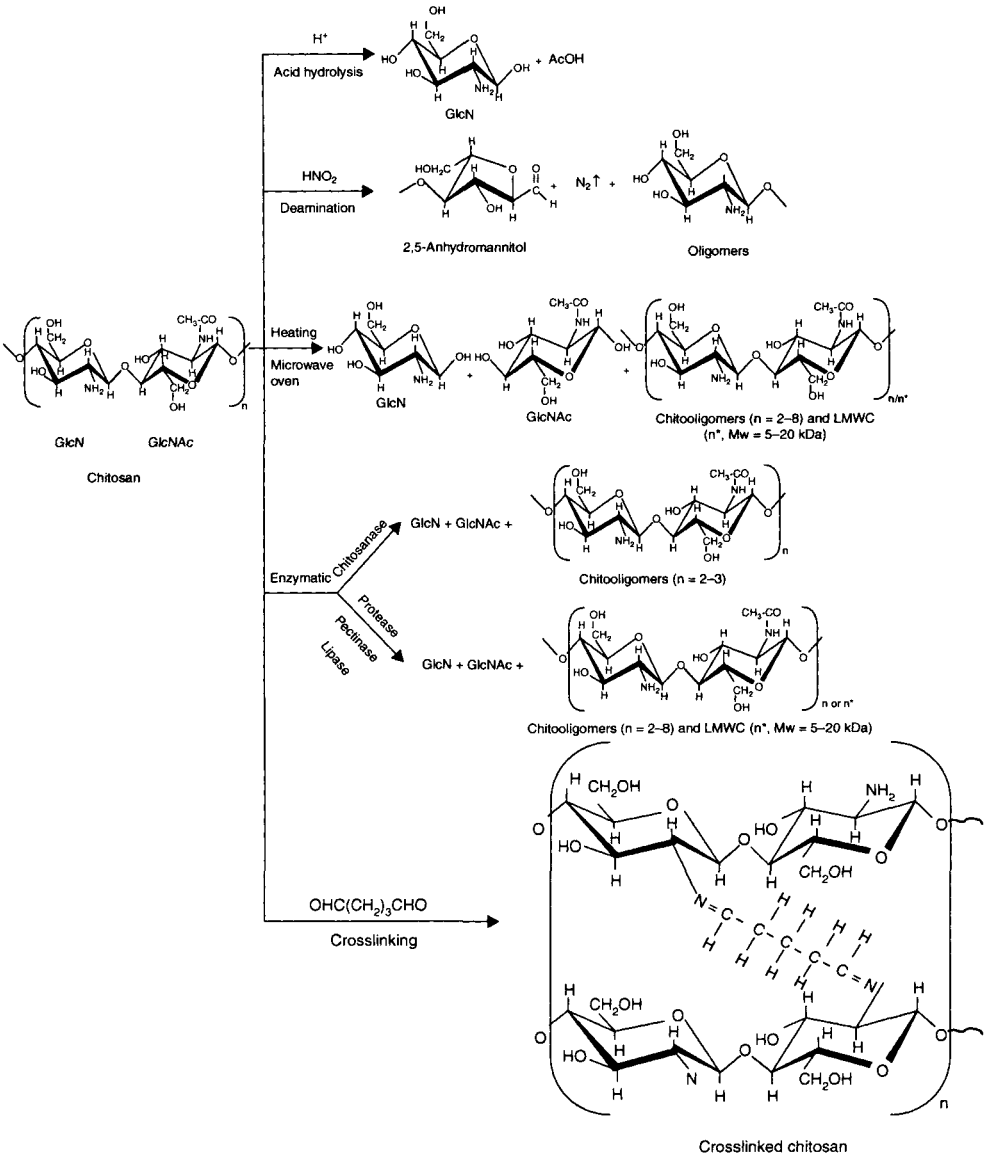


Figure 6.5 Possible depolymerization products and crosslinking of chitosan [1].

nitrous acid and HCl) is a very common and fast method to produce a series of chitooligomers, but the method has some disadvantages such as high cost, low yield and residual acidity. Recently, LMWC have been prepared by salt-assisted acid hydrolysis under microwave irradiation [83]. The mechanism is explained due to the direct absorption of thermal energy by salt molecules, which causes localized superheating of the solution. Increase in the conductivity of the solution as well as dielectric loss and microwave coupling of the solvents have a dramatic influence on the rate of heating. The Mw of chitosan changed drastically in the presence of salt (from 10 x 10^4 to 3 x 10^4 Da),

and the composition of the electrolyte followed the order $K\text{þ} > Ca_2\text{þ} > Na\text{þ}$, which was related to the ionic radius of the metals. Depolymerization of chitosan by the use of nitrous acid (HNO_2) is a homogeneous reaction where the number of glycosidic bonds broken is roughly stoichiometric to the amount of nitrous acid used [84]. The mechanism involves deamination of deacetylated glucosamine residues (D-units) forming 2, 5-anhydro-D-mannose (M-units) at the new reducing end. Since the latter is unstable, the standard procedure has been to reduce to 2, 5-anhydro-D-mannitol by the use of $NaBH_4$. Nitrous acid induced depolymerization has been used previously to study the distribution of Nacetylated units in partially N-acetylated chitosan. It has been found to be specific in the sense that HNO_2 attacks the amino group of D-units, with subsequent cleavage of the adjacent glycosidic linkage. There have been very few reports on the degradation of chitin or chitosan by free radicals [85] that demonstrated that the viscosity of chitosan solution decreased rapidly in the presence of hydrogen peroxide (H_2O_2) and $FeCl_3$, probably because random depolymerization of chitosan [86] showed that Cu(II), ascorbate, and UV-H_2O_2 systems also gradually reduced the molecular weight of chitosan. It has been postulated that the hydroxyl radicals generated in the experimental system caused polymer degradation and that this phenomenon may help to explain the disappearance of chitosan *in vivo* during biomedical applications. There are several other methods to degrade chitosan molecules, including thermal degradation and ultrasonic treatment [87, 88].

6.8.2 Physical Methods

Radiation can provide a useful tool for the degradation of biological polymers and it is often viewed as being the last process after packaging to control pathogenic and spoilage organisms. Recently, radiation effect on carbohydrates such as chitosan, sodium alginate, carrageenan, cellulose, and pectin has been shown to enhance the use for recycling these bioresources and reducing the environmental pollution [89-91]. The irradiation effect on chitosan in acetic acid solution with various dose rates and the yield of chitosan oligomers have been investigated [91]. In another method using 85% phosphoric acid, low molecular weight chitosans were prepared by irradiation at different reaction temperatures and reaction time intervals, wherein the viscosity average molecular weights of chitosan decreased from 21.4×10^4 to 7.1×10^4 after 35 days of treatment [92].

6.8.3 Enzymatic Methods

Although various degradation products of chitosan could be produced by a variety of methods, enzymatic methods are gaining importance because they allow regioselective depolymerization under mild conditions. Nevertheless, the undesirable level of pyrogenicity caused by the presence of protein admixtures in such preparations cannot be discounted. In the case of enzymatic degradation of chitosan, LMWC with high water solubility were produced

by chitinase, chitosanase, glucanase, lipase and some proteases [93-95]. Non-specific enzymes [96], including lysozyme, cellulase, lipase, amylase, papain and pectinase [97, 98] that are capable of depolymerizing chitosan are known, and among these papain, a cysteine protease, is particularly attractive because of its plant origin, wide industrial use in meat tenderization, use in medication for wound debridement, and inhibition by human salivary cystatin.

6.8.4 Graft Copolymerization

The possibility of grafting synthetic polymer to chitosan has attracted much attention in the last years as a new way to modify the polysaccharide and develop practically useful derivatives. Graft copolymerization reactions introduce side chains and lead to the formation of novel types of tailored hybrid materials composed of natural and synthetic polymers. Grafting chitosan is a common way to improve chitosan properties such as formation of inclusion complexes [99], bacteriostatic effect [100], or to enhance adsorption properties [101, 102]. Although the grafting of chitosan modifies its properties, it is possible to retain some interesting characteristics such as mucoadhesivity [103], biocompatibility [104, 105] and biodegradability [106].

Many routes for chitosan grafting have been investigated, such as ring opening polymerization of lactides. Copolymers based on lactic acid have been widely used in sutures and pH-sensitive drug release systems because of their biodegradability. Luckachan *et al.* [107] developed chitosan/oligo(L-lactide) graft copolymers by ring opening polymerisation of L-lactide in DMSO at 90°C under a nitrogen atmosphere using $Ti(OBu)_4$ as catalyst. They obtained a graft copolymer with increased hydrophilicity and controlled degradation rate that may have wide applications in wound dressing and in controlled drug delivery systems. Analogously, Wu *et al.* synthesized a water soluble chitosan derivative grafted with polylactide by ring opening polymerization of D, L-lactide onto chitosan in dimethyl sulfoxide solution, in the presence of triethylamine. The obtained amphiphilic chitosan– polylactide graft copolymer is able to form polymeric micelles. Because of the micelle hydrophobic core they can be used as a promising delivery carrier for the entrapment and controlled release of hydrophobic drugs [108].

Graft copolymerization of vinyl monomers onto chitosan using free radical initiation has attracted the interest of many scientists in the last two decades [109]. Grafting with hydroxyethylmethacrylate (MMA) using azobisisobutironitrile (AIBN) [110], methyl methacrylate using Fenton's reagent as redox initiator [111], dimethylamino ethyl methacrylate and *N,N*-dimethyl-*N*-methacryloxyethyl-*N*-(3-sulfopropyl)ammonium using ceric(IV)salt as redox initiator [112-113], and *N*-isopropylacrylamide by γ–irradiation method [114] have been reported in the literature.

Grafted chitosans have great utility in controlled drug release [115], tissue engineering [116], wound-healing [117] and cardiovascular applications [118-119]. Moreover, there are several reports regarding the use of enzymes in polymer synthesis and modification [120-121]. In fact, enzymes offer the potential advantage

of eliminating the hazards associated with reactive reagents and because of their specificity they offer the potential for precisely modifying macromolecular structure to better control polymer function. Types of chitosan graft copolymers are given in Table 6.2.

6.8.5 Chitosan Crosslinking

The development of procedures to crosslink cationic polysaccharides has increased their applications. Polymer hydrogels with adequate mechanical properties and high drug loading capability show big potential as the basis for controlled drug delivery systems [122]. In the case of chitosan, the amino groups of the polymer may allow the establishment of different types of interactions with both non-ionic and ionic drugs [123] and also provide pH-sensitive systems, which swell in gastric conditions allowing site-specific release [124]. Several cross-linking reagents have been used for crosslinking of chitosan, such as glutaraldehyde, genipin, ethylene glycol, diglycidyl ether and diisocyanate [125-129].

Gupta *et al.* prepared crosslinked chitosan microspheres by dropping a polysaccharide solution into a methanolic solution of NaOH and then adding a solution of glutaraldehyde for crosslinking. They demonstrated that the obtained microspheres were non-toxic and biodegradable, and therefore may be considered suitable candidates for oral drug delivery [130].

The formation of hydrogels from polymers using non-covalent crosslinking is a useful method to prepare hydrogels for drug delivery. These gels are likely to be biocompatible as gel formation does not require the use of organic solvents or chemical reactions, which may be potentially deleterious to the drug load. Such physically cross-linked chitosan-based gels are formed by exploiting either hydrogen bonding or hydrophobic attractions. The use of

Table 6.2 Types of chitosan graft copolymer.

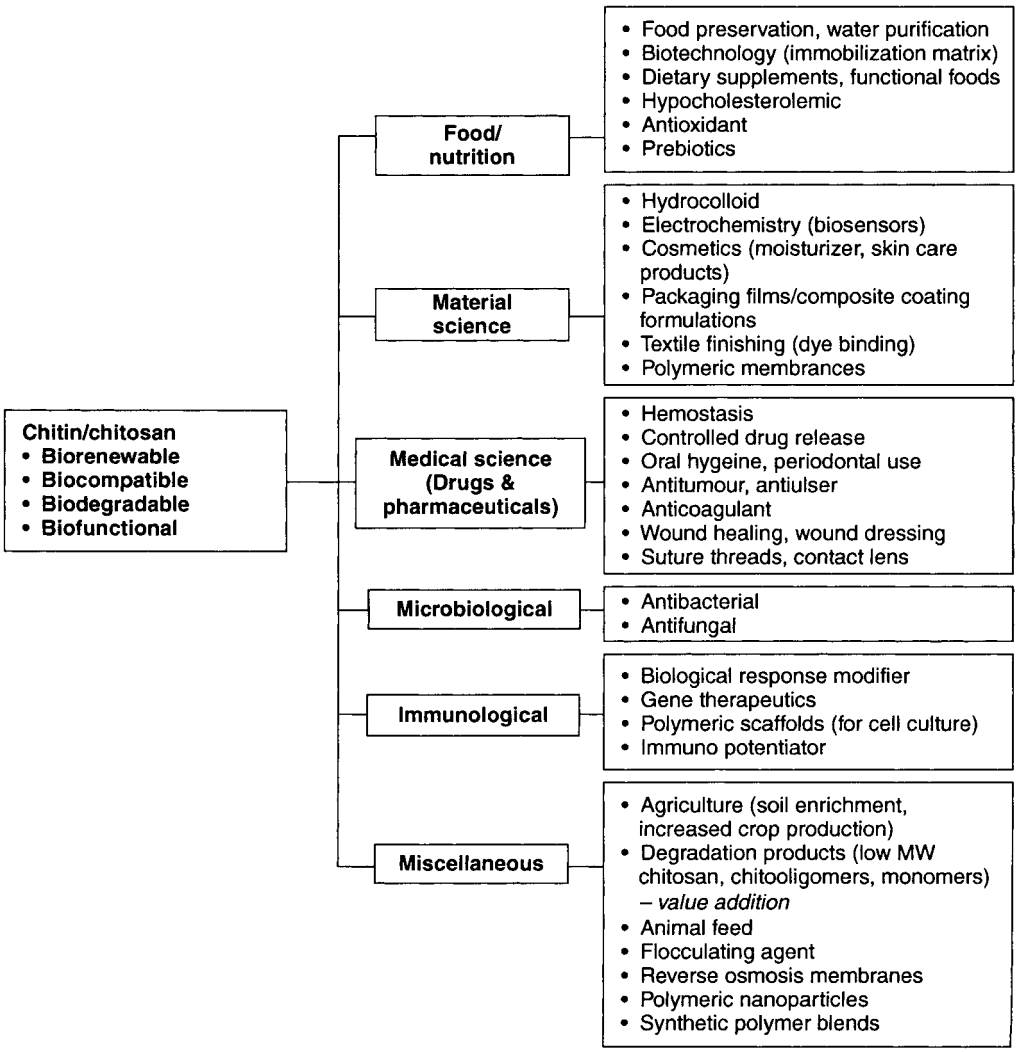
Type	Initiator	Monomers Grafted
Radical-induced	Ce^{4+}, $K_2S_2O_8$, Fenton's reagent (Fe^{2+} + H_2O_2), tributyl borane	Acrylonitrile, N-isopropyl acrylamide, methyl methacrylate, vinyl monomers
Radiation-induced	γ-rays, ^{60}Co	Styrene, 2-hydroxyethyl methacrylate Polyacryl-amide
Microwave irradiation Grafting onto method	Various catalysts are used [4,4'-Azobis (4-cyanovaleric acid)]	Telechelic polymers, polyethylene glycol, poly-dimethyl siloxane
Dendronization	(Reductive *N*-alkylation)	Polyamidoamine, styrene

tripolyphosphate (TPP) in ionic gelation as a polyanion to cross-link with the cationic chitosan through electrostatic interaction could avoid possible toxicity of reagents used in chemical cross-linking (e.g. glutaraldehyde). TPP cross-linked chitosan beads can be prepared simply by dropping chitosan droplets into TPP solution, a procedure that was found to be useful in the pharmaceutical industry [131-134].

6.9 Applications of Chitin and Chitosan

The main applications of chitin and chitosan are summarized below (Table 6.3):

Table 6.3 Applications of chitin and chitosan [1].



6.10 Bio-medical Applications of Chitosan

6.10.1 Gene Therapy

Chitosan is a natural cationic polymer that has recently emerged as an alternative non-viral gene delivery system. Conceptually, gene therapy involves the introduction of an extraneous gene into a cell with the aim of tackling genetic disease, slowing down the progression of tumors and fighting viral infections. Out of the various nonviral vectors tried, such as liposomes, cationic polyelectrolytes, chitosan excels in its biocompatibility and non-toxicity even at escalating doses with *no side effects*.

Due to superior mucoadhesive properties, chitosan facilitates the transport of various drugs across cellular membranes. Chitosan in which over 2 out of 3 monomer units carried a primary amino group formed stable colloidal polyplexes with pDNA. Only the protonated form of soluble chitosan having an uncoiled configuration can trigger the opening of the tight junction zones of DNA, thereby facilitating the paracellular transport of hydrophilic compounds. Chitosan behaved more like a Gaussian coil instead of the worm-like chain model found in other polyelectrolytes. Also the molecular size of chitosan and the pH of the medium are two other important parameters that dictate its permeabilizing and perturbing effects on the cell membrane. A combined electrostatic-hydrophobic driving force from chitosan might induce the destabilization of cell membranes. In addition to ionic interactions, non-ionic interactions between the carbohydrate backbone of chitosan and cell surface proteins might exert an important role in the chitosan-mediated transfection of cells [135].

DNA condensation is a promising means whereby DNA containing genes of therapeutic interest can be prepared for transfer from solution to target cells for gene therapy applications. In addition to synthetic condensation agents (PEG), multivalent cations (chitosan) may also facilitate binding. Such chitosan-based transfection systems are advantageous because of non-immunogenicity, lack of biohazards and the possibility of introducing larger DNA fragments into targets over viral vectors in gene therapy. However, the high molecular weight of chitosan precluded its usage because of toxicity problems. Nevertheless, the LMWCs are neither toxic nor haemolytic and they are shown to form complexes with DNA and protect against nuclease degradation, thereby validating LMWC as components of a synthetic gene delivery system [136].

6.10.2 Enzyme Immobilization

The fact that an enzyme can coexist in various oligomeric forms is of major importance for its catalytic expression. Enzyme immobilization is a technique of significant practical utility, especially when used to enhance catalytic potential, resistance to pH and temperature, and continued reusability. Chitosan is known to be an excellent base material for the immobilization of several carbohydrate degrading enzymes, because it exhibits increased thermostability

compared to the free enzyme. Urease has been immobilized covalently onto glutaraldehyde crosslinked chitosan membrane, especially to provide resistance to the influence of inhibitors, such as boric acid, thioglycolic acid, sodium fluoride and acetohydroxamic acid [137]. Similarly, resistance to mechanical stirring of D-amino acid oxidase (a flavoprotein using FAD as cofactor) has been provided by enzyme immobilization on crosslinked chitosan matrix [138].

6.10.3 Antioxidant Property

The trend to go for potent, naturally derived antioxidant molecules over those of synthetic origin is ever increasing. To this class belong chitosan and several of its derivatives, which being safe and non-toxic offer protection from free radicals, thus retarding the progress of numerous chronic diseases [139]. It is known that the antioxidant effect of chitosan varies with its molecular weight and viscosity, as shown in cooked comminuted fish flesh model systems [140].

This variation is attributed to differences in the availability of net cationic amino groups in the molecule, which impart intermolecular electrostatic repulsive forces leading to an increase in the hydrodynamic volume of the extended chain conformation. The highly unsaturated fatty acids commonly found in seafood are particularly sensitive to oxidative change during storage. Treatment of herring fish samples with chitosan, however, showed lower peroxide values and total volatile aldehydes than the untreated samples. The low viscosity chitosan showed the strongest antioxidative effect [141].

6.10.4 Hypocholesterolemic Activity

Due to its beneficial plasma cholesterol level lowering effect, which plays an important role in the alleviation and treatment of cardiovascular diseases, chitosan has become a useful dietary ingredient. The hypocholesterolemic action of chitosan has been explained as being due to decreased cholesterol absorption and interference with bile acid absorption, a mechanism similar to those of dietary fiber constituents. The cholesterol-lowering action of oral chitosan has been reported by many [142], whereas chitin, although exhibiting a higher excretion of triglycerides in feces, does not display cholesterol-lowering action. Information regarding the digestion and absorption of chitin and chitosan in the GI tract is limited. In an *in vivo* study in a canine GI tract, it was shown that chitin did not undergo any changes in weight and shape, whereas chitosan showed a w10% decrease in weight and formed a film.

6.10.5 Wound-healing Accelerators

The rapid healing of wounds is desirable for patients, especially those suffering from diabetes, because they show an extremely slow rate of healing. Chitin and chitosan have been investigated by many researchers for a long time as possible wound-healing accelerators [143, 144]. Chitin and its derivatives can be applied safely to animals, as well as the humans.

Various forms of chitin-based products are available for medical applications, such as finely divided powder, nonwoven fabrics, porous beads, lyophilized soft fleeces or gels, gauges, laminated sheets, and transparent films. Water-soluble derivatives of chitin in isotonic saline can be administered intramuscularly or intravenously. Certainly, there is considerable biochemical evidence linking NAG with the metabolism of the hexamines, which are assumed to originate and cross-link wound collagen.

The physiological importance of glucosamine has been stressed by various authors: It takes part in the detoxification function of the liver and kidneys and possesses anti-inflammatory, hepatoprotective, antireactive, antihypoxic activities [145-148]. D-Glucosamine in human cancer patients, has also shown natural killer (NK) activity [149]. The repeating monomer subunit of chitin derivatives, NAG, is a major component of dermal tissue, and its presence is essential for the repair of scar tissue. In fact, glycoproteins, which contain large amounts of NAG, are one of the predominant proteins isolated in the early phase of wound healing. It is possible that chitin derivatives, being easily degraded by lysozymes naturally present in wound fluid, could promote wound healing by functioning as a controlled delivery source for NAG to the healing wound [150]. Yano and coworkers showed that chitin promoted wound healing by increasing wound tensile strength during the early stages of wound healing in rat dermal incisions [151]. W. G. Malette, H. J. Quigley, and E. D. Adickes showed that chitosan has a small negative effect on wound tensile strength, which they described as due to inhibition of fibroplastia and subsequent selective tissue regeneration [149]. No abnormal tissue reactions or infections were observed with chitin sutures [150-153]. Similar studies published by Nakajima *et al.* have not supported the original observations of the inhibition of fibroplastia and selective tissue regeneration with chitosan in canine vascular grafts [150].

Chitin is "physiologically" soluble because lysozyme, present in large amounts in healing wounds, acts on it. Chitin derivatives suitable as healing accelerators are formed either with pharmaceutically acceptable groups and esters or salts or with pharmaceutically acceptable acids. Examples are hydroxyethyl chitin, CM-chitin and its zinc salts, methyl chitin, ethyl chitin, chitin acetate, and so on. But, due to differences in physical properties among the chitosan derivatives, they may have different biological properties and interact with healing wounds through different mechanisms. There are several possible mechanisms by which these materials might improve or modify wound healing. A possible mode of action for chitin derivatives in wound healing is one in which glycosaminoglycans play a role in the structural organization of collagen. The glucosaminoglycan constituents of wound tissue play an important role in giving structure and strength to newly formed collagen in the granulating tissue of a healing wound. Studies of chitosan have shown that it does not increase wound tensile strength to the degree that has been shown with chitin. Another mechanism by which chitin derivatives may affect wound healing is their ability to stimulate the inflammatory components of wound healing. It may be that chitin

derivatives or degraded fragments lead to altered functional activity by wound repair cells, which could lead to subsequent wound repair. It is well known that the macrophage cell is central to the healing process [151-154]. The absence of macrophages in macrophage-deficient animals has been shown to lead to impaired wound healing [154]. Chitosan derivatives are hydrolyzed *in vivo* by lysozyme to oligomers that activate macrophages to produce interferon, tumor necrosis factor, and interleukin-1. The oligomer-activated macrophages also produce *N*-acetyl-β-D-glucosamidase, which catalyzes the production of NAG, D-glucosamine, and substituted glucosamines from oligomers. These amino sugars are available to fibroblasts that proliferate under the action of interleukin-1 for incorporation into hyaluronate and glycosaminoglycans, thus guiding the ordered deposition of collagen, also influenced by oligomers. It has already been shown that during tissue regeneration and reorganization processes, chitosan and its derivatives support blood coagulation, prevent abnormal fibroblastic reactivities, and act as a bactericide and wound-healing accelerator [155-157]. The vascularization of newly formed tissue is a very important feature for its physiological functions. The polysaccharides play an active biological role in eovascularization in developing tissue. The ability of chitosan to promote neovascularization has been demonstrated by implanting it in the cornea. It has also been reported that chitosan does not induce other inflammatory events. Recent clinical data indicate that modified chitosans with amino acid moieties and substituents at the *N*-atom, play an active role in wound healing. In general, these induce formation of vascularized and nonrefractive tissues having well-oriented collagen. Modified chitosans depress capsular tissue formation. Certain cartilaginous tissues could also be repaired in view of the angiogenic action of chitosan [158-161].

6.10.6 Artificial Kidney Membrane

Recent developments in the design of artificial kidney systems have made possible repetitive hemodialysis, thereby sustaining the lives of patients with chronic kidney failure. Hemodialysis, an extracorporeal blood purification procedure, utilizes a polymeric membrane to remove the desired amounts of solutes and water from blood. The active part of the artificial kidney is the semipermeable membrane itself, for which commercially regenerated cellulose or cuprophan is still being used due to good solute permeability and mechanical strength. Since the primary action of the cellulose membrane is that of a sieve, there is little selectivity in the separation of two closely related molecules [162]. Chitosan membranes have been proposed as artificial kidney membranes possessing high mechanical strength in addition to permeability to urea and creatinine. These membranes are impermeable to serum proteins. Thus, there is need to develop better hemodialysis membranes to provide greater selectivity and higher dialysis rates for medium- and large-size molecules such as uric acid and the like. A considerable amount of work has been reported in literature either on modification of existing polymeric

membranes or on synthesis of new polymeric membranes to meet the requirement of dialysis applications [163–165].

Chitosan has the potential to be used as an artifical kidney membrane [165]. The film-forming properties of chitosan were studied extensively by several workers, and a variety of chitosan membranes has been proposed (e.g. for reverse osmosis, ion exchange, metal ion uptake, diffusion of dyes, and separation of water-alcohol mixture systems). Chitosan has been modified by graft copolymerization and by blending with water-soluble polymers [166]. Several studies of chitosan/poly(vinyl alcohol) (PVA) blended membranes have been reported [167–169]. According to Miya *et al.* [163], chitosan forms a clear homogenous blend with PVA, and the tensile strength of the blend is greater than the component value. Uragami, Yoshida, and Sugihara [165] prepared a cross-linked chitosan/PVA blend with a fixed amount of cross-linking agent and studied the active transport of the halogen ion through the chitosan/PVA membrane. Kikuchi *et al.* [170, 171] reported about the diffusion of bovine serum albumin in highly cross-linked membrane. It was reported that many polymeric membranes prepared from chitosan derivatives allows the transport of alkali metal ions and lower molecular weight solutes through these membranes [172-176]. In addition, membranes that change the permeation according to simulation such as pH, redox reagent, or light were also recently reported on [173–176]. Kim *et al.* [177] studied a chitosan/PVA blended membrane cross-linked with varying amounts of glutaraldehyde. The permeability coefficients of riboflavin and insulin through these membranes were found to be pH dependent (order of 10^{-6}–10^{-7} cm^2/s). The effect of cross-linking and blending of chitosan with PVA on the permeability of vitamin B-12 were also studied. The partition coefficients, for the membrane studied, were nearly constant ($K \doteq 0.4$), which shows consistency with "pore-type" transport mechanisms for vitamin B-12 [178]. A series of membranes from

the blending of chitosan with PVA and immobilizing bioactive molecules like prostaglandin E1 (PGE1) on heparin have been reported. These membranes show good permeability for small molecules and a dramatic reduction in platelet attachment [179]. Albumin-blended chitosan membranes had superior permeability properties for smaller molecules compared to the standard cellulose membranes, and these membranes showed maximum reduction in platelet adhesion in comparison to other membranes it was reported by some workers [180]. Glycol chitosan membrane modified with thiol groups was investigated for the permeability of KCl and sucrose. The study showed that permeation control can be achieved by varying the amount of cross-linking agent [181]. Hirano *et al.* [182, 183] prepared a series of membranes from chitosan and its derivatives, and the membranes showed improved dialysis properties. They observed that permeability properties of *N*-acetyl chitosan membranes were similar to those of an Amicon Diaflo membrane UM-10 (Amicon Ltd., England). Chitosan membranes were modified with vinyl monomers using ^{60}Co γ-ray irradiation, and their physicochemical properties were also studied. The modified membranes showed improved permeability and blood compatibility [184, 185].

6.10.7 Drug Delivery Systems

The controlled release of drugs is a very important aspect of health care. The potential of chitosan in the design of carriers for the controlled release of drug delivery is enormous in view of its useful properties, like degradability, non-toxicity, and biocompatibility. The degraded products of chitosan do not introduce any disturbance in the body. Hence, it can be a suitable matrix, available in different forms, for the sustained release of various drug formulations. Various drugs may well be incorporated into chitosan matrix in a variety of forms (namely, beads, films, microcapsules, coated tablets, etc.) for controlled release therapies. Drugs incorporated into chitosan, in different forms, are listed in Table 6.4 [186-212]. A zero-order release for water-soluble drugs is reported for chitosan-coated tablets. The release profile of a drug depends on various parameters, like initial drug loading, thickness of membrane, pH of buffer system, and type of mechanisms involved [213]. The release of mitoxantrone

Table 6.4 Chitosan based drug delivery systems.

Drug	Dosage Form
Chlorpheniraniine maleate (CPM)	Tablet
Aspirin	Wet granulation formulation
Prednisolone	Granules
Methamphetamine (MA)	Body spacer
5-Fluorouracil (5FU)	Conjugate prodrug
Propranolol hydrochloride	Tablet
Griseofulvin (GRF)	Tablet
Phenytoin (DPM)	Tablet
Diazepam	Film (coating)
Cisplatin	Microsphere
Brilliant blue	Tablet
Coomassie brilliant blue	Film
Pullulan	Film
Cisplatin	Microsphere
Griscofulvin	Spherical agglomerates
Ibuprofen	Spherical agglomerates
Idomethacin	Spherical agglomerates

Table 6.4 (cont.) Chitosan based drug delivery systems.

Drug	Dosage Form
Sulfadiazine	Spherical agglomerates
Tolbutamide	Spherical agglomerates
Oxyphenbutazone	Coated tablet
Glafenine	Coated tablet
Nefedipine	Microgranules
Diclofenac sodium	Tablet
Chlorhexidine acetas	Semi-PIN gel disk
Interleukin-2	Microsphere
Nefedipine	Membrane
2′- or 3′-(4-Carboxy-butyryl-5-fluorouridine)	Microsphere
Clofibrate	Coacervates
Piroxicam	Coacervates
Sulfamethyoxyzole	Coacervates
Nefedipine	Microsphere
Indomethacin	Conjugates
Insulin	Coating
Prednisolone sodium phosphate	Microsphere
Amoxicillin	Semi-IPN hydrosel
Metronidazole	Semi-IPN hydrogel
Diclofenac sodium	Gel beads
Flurbiprofen	Gel beads
Dapsone	Gel
Bromthymol blue	Gel
Mitoxantrone	Microsphere

from glutaraldehyde cross-linked chitosan microspheres was studied *in vitro* in phosphate buffer for over 4 weeks at 27 °C. Only about 25% of the incorporated drug released over 36 days from microspheres of high cross-linking density. Histological analysis showed that the microspheres were tolerated by the living tissue [214]. Controlled release of bovine albumin and interleukin-2

were studied from alginatechitosan microspheres. Sustained release of proteins from microspheres was of longer duration than from other microsphere formulations. Interleukin released in a sustained manner triggered the induction of cytotoxic T lymphocytes more efficiently than dried free interleukin [215]. The transdermal release of nifedipine and propranolol hydrochloride from collagen-chitosan composite membranes was studied. About 9.5 mg of nifedipine and 5 mg of propranolol chloride were released from the patches in a near-zero-order fashion over 24 h after a short initial lag time [216]. The acid stability and physiochemical properties of glutaraldehyde cross-linked chitosan microspheres containing prednisolone sodium phosphate were studied. Microsphere size and acid stability increased with increasing cross-linking agent concentrations, whereas drug loading was reduced. Acid stability and loading efficacy decreased with increasing reaction time; size was affected by reaction time [217].

For localized antibiotic delivery in the stomach, a pH-sensitive swelling system was synthesized by cross-linking chitosan and polyethylene glycol poly(ethylene oxide) (PEO) in a blend form. From these semi-interpenetrating polymer networks (semi-IPNs), the release of amoxicillin and metronidazole in enzyme-free simulated gastric fluid (SGF) and simulated intestinal fluid was studied. More than 65% of amoxicillin and 59% of metronidazole were released from freeze-dried chitosan-PEO semi-IPNs after 2 h in SGF. These results suggest that freeze-dried semi-IPNs could be useful for localized delivery of antibiotics in the acidic environment of gastric fluid [218]. A pendant type of polymeric drug was shown to release an active drug through two-step hydrolysis by a dual enzyme system when biodegradable CM-chitin was used as a carrier. The two-step release of the active drug was also suggested for the prodrug, which is absorbed onto the CM-chitin Ca_2 complex. The main factor in regulating the above phenomenon is stabilization of drugs under physiological conditions so that they resist the enzymatic attack. Recently, chitosan-5-fluorouracil (5FU) conjugate was investigated to provide water-soluble macromolecular prodrug of 5FU with reduced side effects, affinity for tumor cells, and strong antitumor activity. High susceptibility of CM chitin by lysozyme in the animal body permits its applications as a drug carrier for practical drug delivery systems [219].

6.10.8 Blood Anticoagulants

Heparin, one of the most widely used blood anticoagulants, is an expensive product. Attempts have been made to prepare a number of synthetic anticoagulants, but none are as nontoxic as heparin. It has been reported that cellulose and starch sulfuric acid esters are toxic, whereas chitin disulfuric acid is less toxic. It has also been reported that the protein moiety of chitin is responsible for the inflammatory response when material containing chitin is injected into tissues of higher animals, whereas pure chitin does not give a detectable response. Dutkiewicz *et al.* [218] have shown that chitosan exhibits not only a hemostatic effect, but also has anticoagulant properties. Some variation

reported in the literature regarding thrombogenicity of chitosan may be due to the difference in origin, degree of deacetylation, molecular weight, and/or other characteristics of chitosan used by various researchers. Chitin is a suitable starting material for the production of heparin-like blood anticoagulants. Sulfonation of chitosan has been one of the most attractive modifications owing to the possibility of preparing anticoagulant polysaccharides in view of the structural similarity with heparin. For sulfonation, various reagents have been used by many researchers [219-222]. Sulfonated derivatives of chitosan possess blood anticoagulant activity. Conversion of position 6 into a carboxyl group in *N*-sulfonated chitosan creates a product with 23% of the activity of heparin [223], and its *O*-sulfonated form exhibited 45% activity *in vitro* [224]. It has been reported that, as the content of sulfur increases in chitosan, the anticoagulant activity of sulfonated chitosan increases. *N*-Carboxymethyl chitosan 3,6-disulfonate of low molecular weight exhibited anticoagulant activity similar to that of heparin and showed no adverse effects on the cellular structures when added to blood [225]. This may be useful in storing blood. The knowledge of molecular weight is a prerequisite for the preparation of products with a reproducible degree of sulfonation and consequent biochemical characteristic properties. The assessment of applicability of these substances *in vivo* will be possible only when the chemistry necessary to produce them is fully understood.

6.10.9 Artificial Skin

Chitosan with structural characteristics similar to glycosaminoglycans could be considered for developing a skin replacement. Yannas and Burke [226] proposed a design for artificial skin applicable to long-term chronic use, with the focus on a nonantigenic membrane that performs as a biodegradable template for neodermal tissue. Kifune, Yamaguchi, and Kishimoto [227] recently developed a new wound dressing material, Beschitin W, a commercial product that is composed of chitin nonwoven fabric and that has been found to be beneficial in clinical practice. Kim and Min [228] have developed a wound covering material from polyelectrolyte complexes of chitosan and sulfonated chitosan. Wound healing is accelerated by oligomers of degraded chitosan by tissue enzymes, and this material was found to be effective in regenerating the skin tissue of the wound area. The pharmaceutical company Katakurachikarin, based in Hokkaido, Japan, makes an artificial skin (a chitosan-collagen composite) that appears to enhance recovery from surgical wounds or burns.

6.11 Miscellaneous Applications

Chitosan and its amino acid derivatives (poly D, L-lactic acid) have been explored as an extracellular matrix-like surface to promote cell adhesion and growth [229]. Four kinds of chitosan-amino acid derivatives were prepared to minimize the carbohydrate moieties of cell matrix glycoproteins.

From detailed cell cultural studies these chitosan derivatives were shown to promote chondrogenesis. Chitosan nanoparticles are shown to enhance oral bioavailability and intestinal absorption of peptide and protein formulations. By ionotropic gelation of chitosan with tripolyphophate anions insulin-loaded nanoparticles have been prepared [230].

Enhanced intestinal absorption as well as the relative increase in the pharmacological bioavailability of insulin was investigated by monitoring the plasma glucose level of alloxan-induced diabetic rats. The nanoparticle, having a size in the range of 250-400 nm and polydispersity index < 0.1, positively charged and remaining stable, showed an insulin association of over 80%, and its *in vitro* release showed a great initial burst with a pH-sensitivity. It showed an increased absorption of insulin *in vivo* (w15%), and the hyperglycemia was prolonged for over 15 h. Chitosan-poly(acrylic) acid polyionic complexes have been prepared for prolonged gastric antibiotic delivery [231].

Different polyionic complexes of amoxicillin, chitosan and polyacrylic acid were prepared, and employing a non-invasive method the gastric residence time of the formulations was evaluated by means of a 13C-octanoic acid breath test. All the complexes showed extensive swelling, and diffusion of the antibiotic was controlled by the degree of polymer-drug interaction. In the construction of heart valve substitutes, bovine pericardium fixed in buffered glutaraldehyde is presently being used. Calcification limits the durability of such heart valve substitutes. As an alternative, crosslinking of biomacromolecules with glutaraldehyde was tried, which creates void spaces in the fiber matrix leading to exposure of potential binding sites for calcification. By hydrogen peroxide degradation LMWC (2000 Da) was prepared for coupling onto polymer grafted glutaraldehyde crosslinked pericardial tissue to prevent calcification in a rat subcutaneous model [232].

The capacity to preconcentrate anions has enabled the use of chitosan and its derivatives in modified electrodes, for application in sensor and biosensor electrochemistry [233]. To facilitate improving water solubility of biologically useful chitosan derivatives, N-methylene phosphonic chitosan has been prepared using a one-step reaction that allowed homogeneous modifications [234].

The resulting NH_2-CH_2-$(PO_3)_2$ combines its strong donor effect with a monodentate ligand as $(PO_3)_2$, thus increasing its metal-binding properties, especially for calcium. The derivative also shows good filmogenic nature. Phosphated chitin (P-chitin) has been used as an anti-inflammatory agent in a mice model of chitosan-induced acute respiratory distress syndrome [235]. The interstitial pneumonia was thus successfully blocked by a simultaneous intravenous injection of P-chitin. Intravenous infusion of some P-chitin formulations dramatically reduced lung injury and diminished the accumulation of neutrophils in the interstitial and alveolar spaces of the lungs. P-chitin with a Mw of 24000, DS of 58 sulfation at C-6 seems to decrease the anti HIV-1 activity, but nevertheless the latter showed high anticoagulant activity. These new inhibitors of retrovirus infection showed both low cytotoxicity and low anticoagulant activity.

Chitooligosaccharides stimulate purportedly beneficial gut species (Bifidobacterium and Lactobacillus sp.) opening up the possibility of them acting as prebiotics. Despite this property, in a mixed culture system no increase in the bifidus counts was observed [236]. Nevertheless, through pure culture studies chitooligosaccharides were shown to be stimulatory to Bifidobacterium bifidum and Lactobacillus sp.; in low concentrations they led to increased cell numbers and showed prebiotic effects [237]. *In vitro* studies have shown that chitooligosaccharides can bind 4–5 times its weight of micellar lipids, leading to some brands of diet pills claims of fat adsorption, and therefore is of use in controlling obesity [238].

6.12 Antimicrobial Properties

The antimicrobial property of chitosan and its derivatives, with conflicting results, has received considerable attention in recent years due to the imminent problems associated with synthetic chemical agents. Such an application stems from the cationic charge of chitosan molecule to give rise to aggressive binding onto the microbial cell surface, leading to gradual shrinkage of the cell membrane and finally the death of the cell. Several possible explanations have been proposed for antimicrobial activity, namely, the polycationic chitosan molecule interacting with the predominantly anionic cell wall components (lipopolysaccharides and proteins) of the microorganism, which results in the leakage of intracellular components due to changes in the permeability barrier; preventing nutrients from entering the cell; upon entry into the cell (especially LMWC), binding to DNA, and thus inhibiting RNA and protein synthesis; binding through hydrophobic interactions, etc. Chitosan shows a broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria and fungi [239].

In a study on the mode of the antimicrobial action of chitosan (250 ppm at pH 5.3) by monitoring the uptake of the hydrophobic probe 1-N-phenylnaphthylamine, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhimurium showed significant uptake which was reduced (in coli, Salmonellae) or abolished (aeruginosa) by $MgCl_2$. Chitosan also sensitized P. aeruginosa and Salmonellae to the lytic effect of sodium dodecyl sulfate. Electrophoretic and chemical analyses of the cell-free supernatants revealed no release of LPS or other membrane lipids. Electron microscopic observations showed that chitosan caused extensive cell surface alterations and covered the outer membrane with vesicular structures, resulting in the loss of the barrier functions [240].This property of chitosan is useful in food preservation and food protection. To enhance the antibacterial potency of chitosan, thiourea chitosan was prepared by reacting chitosan with ammonium thiocyanate followed by its complexing with Agþ [241].

It has been reported that quaternary ammonium salt of chitosan exhibits good antibacterial activities, for example, diethylmethylchitosan chloride showed higher antibacterial activity than chitosan. Novel N,O-acyl chitosan

derivatives were more active against the gray mould fungus Botrytis cinerea and the rice leaf blast fungus Pyricularia oryzae; hydroxypropyl chitosan grafted with maleic acid sodium killed over 99% of Staphylococcus aureus and E. coli within 30 minutes of contact at a concentration of 100 ng/ml; hydroxypropyl chitosan was a potent inhibitor of Azatobacter mali, Clostridium diplodiella, Fusarium oxysporum and Pyricularia piricola [241].

The degree of substitution of the hydroxypropyl group also influenced their antifungal activity. With regard to their antifungal mechanisms, it was reported that these chitosan derivatives directly interfered with fungal growth and activated several defense processes, such as accumulation of chitinases, synthesis of proteinase inhibitors and induction of callous synthesis. It was also noted that the antibacterial activity of chitosan derivatives increased with increasing chain length of the alkyl substituent, and this was attributed to the increased hydrophobicity [241].

6.13 Film-forming Ability of Chitosan

Chitosans with higher molecular weight have been reported to have good film-forming properties as a result of intra- and intermolecular hydrogen bonding. A patent was granted to G.W. Rigby in 1936 for the earliest attempt to form films from chitosan. These films were described as flexible, tough, transparent, and colorless with a tensile strength of about 9,000 psi and prepared by a solvent casting method. The films were also described as having good gas barrier and mechanical properties [242-246].

The chitosan film characteristics, however, varied from one report to another. Differences in the sources of chitin used to produce chitosan, chitosan properties, solvents used, methods of film preparation, and types and amounts of plasticizers used affected the quality of the chitosan films [247, 248]. The film-forming ability of chitosan extracted from crawfish has also been reported [249].

6.14 Function of Plasticizers in Film Formation

Films prepared from pure polymers tend to be brittle and often crack upon drying. Addition of food-grade plasticizers to film-forming solution alleviates this problem [250].When a plasticizer is added, the molecular rigidity of a polymer is relieved by reducing the intermolecular forces along the polymer chain. Plasticizer molecules interpose themselves between the individual polymer chains, thus breaking down polymer-polymer interactions, making it easier for the polymer chains to move past each other. The plasticizer improves flexibility and reduces brittleness of the film. Polyethylene glycol, glycerol, propylene glycol, and sorbitol are the most commonly used plasticizers in edible film production [251].

The amount of plasticizer added can cause adverse effects on film properties such as increasing mass transfer through the films. Hence, plasticizers must

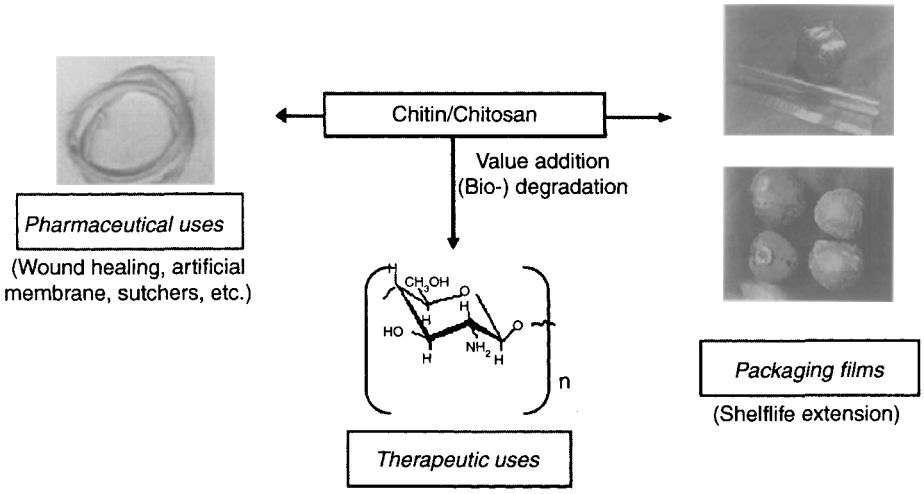


Figure 6.6 Production of and value addition to chitin/chitosan [1].

be used with caution. When the plasticizer concentration exceeds its compatibility limit in the polymer, it causes phase separation and physical exclusion of the plasticizer. This leads to development of a white residue on edible films which have been referred to as "blooming" or "blushing" [252]. The amount of plasticizer used in film formation should also be small enough to avoid any probable toxic effects [253].

6.15 Membranes

Chitosan and several of its innumerable derivatives have the ability to form thin membranous films of use in packaging [254-256], encapsulation and drug delivery systems. Due to drug polymer interactions, high viscosity chitosan films showed better sustainable release; and the mechanism of release followed Fickian diffusion control with subsequent zero order release [257].

A novel organic (chitosan) and inorganic (tetraethyl orthosilicate) composite membrane has been prepared, which is pH sensitive and drug permeable [258]. The latter possibly involved in ionic interactions. By plasma source ion implantation technique, the adhesion between linear low-density polyethylene and chitosan could be improved [259]. Such bilayer films showed 10 times lower oxygen permeability, a property of use in food packaging applications. These multilayer films were easily recyclable.

6.16 In Wastewater Treatment

The prime commercial applications for chitosan currently is in industrial wastewater treatment (Table 6.5) since chitosan carries a partial positive charge and binds to metal ions, thus making the metal ions removal from waste

Table 6.5 Applications of chitosan.

Wastewater Treatment	Removal of metal ions, flocculant/coagulant, protein, dye, amino acids
Food Industry	Removal of dye, suspended solids, preservative, color stabilization, food stabilizer, thickener and gelling agent, animal feed additive, etc.
Medical	Wound and bone healing, blood cholesterol control, skin burn, contact lens, surgical sutures, dental plaque inhibition, clotting agent, etc.
Agriculture	Seed coating, fertilizer, controlled agrochemical release
Cosmetics	Moisturizer, face, hand, and body creams, bath lotion, etc.
Biotechnology	Enzyme immobilization, protein separation, cell recovery, chromatography

streams or contamination sites easier [260]. In terms of utilization, crawfish chitosan as a coagulant for the recovery of organic compounds in wastewater was demonstrated to be equivalent or superior to, the commercial chitosans from shrimp and crab waste shell and synthetic polyelectrolyte in turbidity reduction [261, 262].

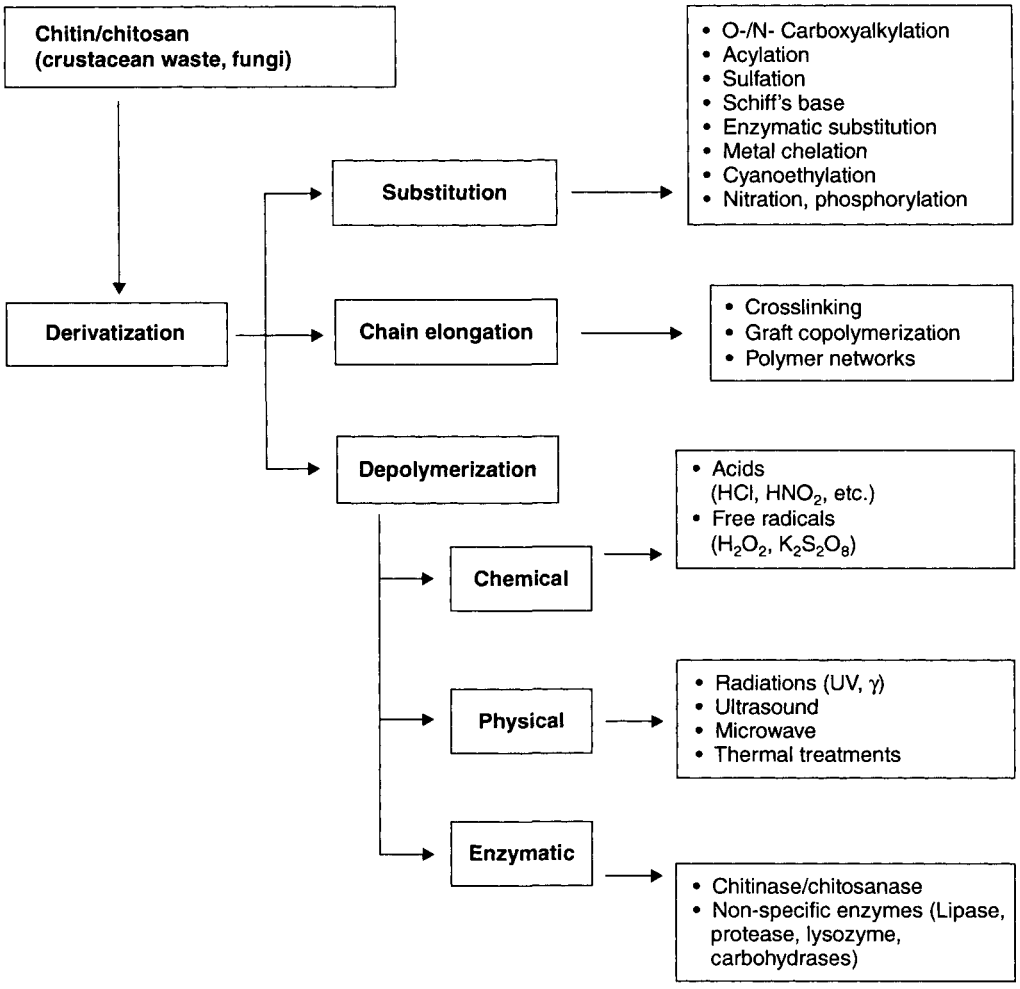
The wastewater released from food processing plants, typically from the seafood, dairy or meat processing industries, contains an appreciable amount of protein which can be recovered with the use of chitosan; this protein, after drying and sterilization, makes a great source of feed additives for farm animals [262].

The removal of dyes is difficult to achieve because of their high resistance to degradation by light, chemical, biological, and other exposures. However, chitin and chitosan have been found to have an extremely high affinity for dyes which may contribute to aquatic toxicity. It was also found that chitosan is effective for conditioning municipal and industrial sludge due mainly to their effectiveness in sludge conditioning, rapid biodegradability in soil environments, and economic advantages in centrifugal sludge dewatering [263].

6.17 Multifaceted Derivatization Potential of Chitin and Chitosan

The multifaceted derivatization potential of chitin and chitosan is summarized in Table 6.6.

Table 6.6 Multifaceted derivatization potential of chitin and chitosan [1].



6.18 Conclusion

Chitosan is a biodegradable, biocompatible, non-toxic and very abundant marine-based natural polymer. It has enormous applications in different branches of modern science such as the nanobiotecnology, biotechnology, biomedical and pharmaceutical fields.

The aim of this chapter was to present an overview of the state-of-the-art knowledge and technical applications of chitin and chitosan. Numerous papers have been published on a wide range of the properties and applications of chitin and chitosan, forcing us to make a selection from the most significant results obtained by many groups working around the world; therefore, we have included an extensive bibliography of recent studies of both basic and applied chitosan, the most important derivative of chitin, which is difficult to

obtain a reproducible initial polymer of because controlling the distribution of acetyl group along its backbone is problematic. Unlike chitin, chitosan is soluble in weak acid or under precisely specified conditions at neutral pH, allowing much development in the domains of solutions and hydrogels. The structure of chitosan allows specific modification without too many difficulties at the C-2 position. This is the main advantage of chitosan over other natural polysaccharide like cellulose, starch, etc. Hence, the natural biological properties of chitin and chitosan are valuable for both plant and animal applications, and such developments can be considered as valuable extensions of the use of chitin and its derivatives. That is why more attention and research is needed to make the world aware of this "Wonder Polymer."

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PART 2
BIOPLASTICS AND BIOCOMPOSITES

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (3–34)

7

Biopolymers Based on Carboxylic Acids Derived from Renewable Resources

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Abstract

Biodegradable polymers are used in an increasingly large number of mass-produced applications such as packaging, paper coating, fibers, films, and other disposable articles, as well as in biomedical applications such as restorable surgical sutures, implants, and controlled drug delivery devices. Among possible monomers, lactic acid and glycolic acid, which are non-toxic, naturally occurring, and renewable raw materials, fulfill most of these rigorous requirements. Since the chemical synthesis is unfriendly to the environment and has more impurities, there has been a continued interest in a more efficient fermentation process for the production of carboxylic acid, its recovery and purification. Reactive extraction with a specified extractant has been proposed as a promising technique for the recovery of acids. Polymers such as poly(lactic acid), poly(glycolic acid) and their copolymers [poly(lactic/glycolic acids)] are produced from these renewable bio-derived monomers. This chapter mainly focuses on and presents a state-of-the-art review of various techniques to produce lactic- and glycolic acid, their recovery from fermentation broths, and applications as biopolymers. It also covers recent polymerization techniques to produce low and high-molecular-weight biopolymer, which has tremendous applications.

Keywords: Carboxylic acids, poly(lactic acid), poly(glycolic acid), fermentation technology, reactive extraction, polymerization

7.1 Introduction

The field of biopolymers, while still in its early stages, is growing in popularity every day. Biopolymers are the polymers which are generated from renewable natural sources, and are often biodegradable and nontoxic. They can be produced by biological systems (microorganisms, plants and animals), or chemically synthesized from biological materials such as sugars, starch, natural fats or oils, etc. Strategies applied in converting these raw materials into biodegradable polymers are: (1) Extraction of the native polymer from a plant or animal tissue, and (2) a chemical or biotechnological route of monomer polymerization.

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (169–182)

Biodegradable biopolymers (BDP) are an alternative to petroleum-based polymers (traditional plastics). It will be important to find durable plastic substitutes, especially in short-term packaging and disposable applications. The continuously growing public concern concerning this problem has stimulated research interest in biodegradable polymers as alternatives to conventional non-degradable polymers such as polyethylene and polystyrene, etc. The economic value of renewable raw materials will increase to a significant extent [1] and induce new industrial activities [2, 3].

Polymers derived from renewable resources (biopolymers) are broadly classified according to the method of production: (1) Polymers directly extracted/removed from natural materials (mainly plants) (e.g. polysaccharides such as starch and cellulose and proteins such as casein and wheat gluten), (2) polymers produced by "classical" chemical synthesis from renewable bio-derived monomers [e.g. poly(lactic acid), poly(glycolic acid) and their biopolyesters polymerized from lactic/glycolic acid monomers, which are produced by fermentation of carbohydrate feedstock] and (3) polymers produced by microorganisms or genetically transformed bacteria [e.g. the polyhydroxyalkanoates, mainly poly(hydroxybutyrates) and copolymers of hydroxybutyrate (HB) and hydroxyvalerate (HV)] [4].

Biodegradable polymers are used in an increasingly large number of mass produced applications such as packaging, paper coating, fibers, films, and other disposable articles, as well as in biomedical applications. Particularly in the packaging sector, the raw materials should be annually renewable and the end products should be compostable in order to reduce the use of fossil resources. Among possible monomers, lactic acid (2-hydroxy propionic acid) and glycolic acid (hydroxyl acetic acid), which are non-toxic, naturally occurring, and renewable raw materials, fulfill most of these rigorous requirements. Therefore, in this chapter, we will focus on the type 2 biopolymers (polymers produced by "classical" chemical synthesis from renewable bio-derived monomers). We will also discuss synthesis of both the monomers (lactic acid and glycolic acid) and their recovery from fermentation broths, and the synthesis of biopolymers based on these bio-derived monomers. The applications of these biopolymers will also be covered.

7.2 Carboxylic Acids: Lactic- and Glycolic Acid

Lactic acid (2-hydroxypropanoic acid), also known as milk acid, is a chemical compound that plays a role in several biochemical processes. Lactic acid is chiral and has two optical isomers. One is known as L-(+)-lactic acid and the other, its mirror image, is D-(-)-lactic acid as shown in Figure 7.1. L-(+)-Lactic acid is the biologically important isomer. Lactic acid is soluble in water and water miscible organic solvents but insoluble in other organic solvents. Except for the applications of lactic acid in the biopolymer industries, it is also used as an acidulant/flavouring/pH buffering agent or inhibitor of bacterial spoilage in a wide variety of processed foods. The addition of lactic acid aqueous

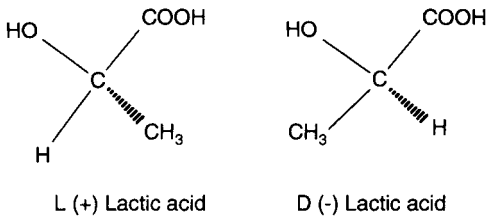

Figure 7.1 Two isomers of lactic acid.

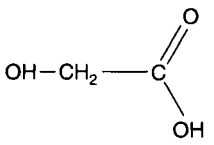

Figure 7.2 Structure of glycolic acid.

solution to the packaging of poultry and fish increases their shelf life [5]. Lactic acid has many pharmaceutical and cosmetic applications and formulations in topical ointments, lotions, anti-acne solutions, humectants, parenteral solutions and dialysis applications.

Glycolic acid (2-hydroxyethanoic acid) is the simplest and smallest organic molecule of the α-hydroxy acid family of carboxylic acids with both the acid (-COOH) and alcohol (-OH) functionality (Figure 7.2).

Glycolic acid occurs naturally as a trace component in sugarcane, beets, grapes and fruits. The acid has a broad spectrum of consumer and industrial applications including use in water well rehabilitation, in the leather, oil, gas, laundry and textile industries, and as a component in personal care products such as skin care creams. It is used as a monomer in the preparation of polyglycolic acid and other biocompatible copolymers (e.g. copolymer of lactic- and glycolic acid). Glycolic acid is often included in emulsion polymers, solvents, and additives for ink and paint in order to improve flow properties and impart gloss. Copolymers can be made with other alpha hydroxy acids like lactic acid. The polyester polymers gradually hydrolyze in aqueous environments at controllable rates. This property makes them useful in biomedical applications such as dissolvable sutures and in applications where a controlled release of acid is needed to reduce the pH.

7.2.1 Lactic- and Glycolic Acid Production

Lactic- and glycolic acids can be manufactured by (a) chemical synthesis or (b) carbohydrate fermentation.

7.2.1.1 Chemical Synthesis

The commercial process for chemical synthesis of lactic acid is based on lactonitrile. Hydrogen cyanide is added to acetaldehyde in the presence of a base to produce lactonitrile. This reaction occurs in the liquid phase at high atmospheric

pressures. The crude lactonitrile is recovered and purified by distillation, and then hydrolyzed to lactic acid, either by concentrated HCl or by H_2SO_4 to produce the corresponding ammonium salt and lactic acid. Lactic acid is then esterified with methanol to produce methyl lactate, which is removed and purified by distillation and hydrolyzed by water under acid catalyst to produce lactic acid and the methanol, which is recycled. The chemical synthesis method produces a racemic mixture of lactic acid. Two companies Musashino, Japan and Sterling Chemicals, Inc., USA use this technology. Glycolic acid is prepared by the reaction of chloroacetic acid with sodium hydroxide followed by re-acidification. The net reaction can be summarized as follows:

$$ClCH_2CO_2H + NaOH \quad HOCH_2CO_2H + NaCl$$

7.2.1.2 *Fermentation Process*

Though chemical synthesis produces a racemic mixture, stereo specific acid can be made by carbohydrate fermentation depending on the strain being used. Total fermentation is described by two processes: (a) Fermentation and neutralization of carbohydrates, and (b) purification or separation of lactic acid from the broth. The desirable characteristics of industrial microorganisms are: (1) They have the ability to rapidly and completely ferment cheap raw materials, (2) they require a minimal amount of nitrogenous substances, (3) they provide high yields of preferred stereo specific lactic acid under conditions of low pH and high temperature, (4) they produce low amounts of cell mass, and (5) they produce negligible amounts of other byproducts. The choice of an organism primarily depends on the carbohydrate to be fermented. For example, *Lactobacillus delbreuckii* is able to ferment sucrose; *Lactobacillus delbreuckii* is able to use lactose; *Lactobacillus helveticus* is able to use both lactose and galactose; *Lactobacillus lactis* can ferment glucose, sucrose and galactose.

Glycolic acid can be produced via fermentation process [6] from glycolonitrile hydrolysis by mineral acid, such as sulfuric acid [7,8]. Both processes produce a multi-component solution with the acid concentration typically less than 10 wt% for fermentation technology, and less than 40 wt% for glycolonitrile hydrolysate. The acid can be produced by the enzymatic conversion (typically the enzyme catalyst used is nitrilase or a combination of a nitrile hydratase and an amidase) of glycolonitrile which results in the production of an aqueous solution of ammonium glycolate [9].

$$HO-CH_2-CN \xrightarrow{Nitrilase,\ H_2O} HO-CH_2-COOH + NH_3$$

7.2.1.3 *Separation of Lactic- and Glycolic Acid from Fermentation Broth*

In recent years, the interest in lactic- and glycolic acid recovery from fermentation broth has increased. This interest is due to an increase in the demand for pure, naturally produced lactic- and glycolic acid, mainly for the food industry

(as a food additive and preservative), pharmaceutical industry, or for the production of biodegradable polymers. The classical approach involves acid precipitation with calcium hydroxide. After filtration, the precipitate is treated with sulphuric acid resulting free acid and calcium sulphate. In the calcium precipitation process, the separation and final purification stages account for up to 50% of the production costs and produce a large quantity of solid waste. Among the various available alternate processes for simultaneous removal of the product, extraction is often the most suitable one. Reactive extraction with a specified extractant which gives a higher distribution coefficient has been proposed as a promising technique for the recovery of carboxylic and hydroxycarboxylic acids. Reactive liquid-liquid extraction has the advantage that acid can be removed easily from the fermentation broth, preventing the lowering of the pH. Due to the use of a high concentration substrate as the process feed, process waste and production cost can be reduced. Furthermore, acid can be re-extracted and the extractant can be recycled into the fermentation process. Among the various extractants, aminic extractants have been extensively studied because of their high efficiency and selectivity. Tertiary amines and quaternary ammonium salts have been found to be better choices than primary and secondary amines. Lactic acid fermentation is found to be end-product inhibited by an un-dissociated form of lactic acid. Several studies have been carried out to overcome this problem. It was discovered that the extractive lactic acid fermentation technique could produce a lactic acid yield of 0.99g/l and a productivity of 1.67 g/l/h over a conventional batch reactor which produced a yield of 0.83 g/l and productivity of 0.31 g/l/h [10].

7.3 Polymerization of Lactic- and Glycolic Acids

7.3.1 Polymerization of Lactic Acid

Poly(lactic acid) can be prepared from lactic acid and/or from one or more of D-lactide (i.e. a dilactone, or a cyclic dimer of D-lactic acid), L-lactide (i.e. a dilactone, or a cyclic dimer of L-lactic acid), meso D, L-lactide (i.e. a cyclic dimer of D-, and L-lactic acid) and racemic D, L-lactide (racemic D, L-lactide comprises a 1:1 mixture of D-, and L-lactide). Lactic acid forms PLA upon polycondensation (Figure 7.3). A disadvantage of polycondensation is that a low molar mass polymer is obtained. Lactic acid is also oligomerized and then catalytically dimerized to make the monomer for ring-opening polymerization (Figure 7.3). It can be easily produced in a high molecular weight form through ring-opening polymerization most commonly by the use of a stannous octoate catalyst. However, the cost of producing PLA using the ROP method is high due to the complicated purification process of the lactide. In 1995 Ajioka *et al.* [11] developed a direct solution poly-condensation process that was able to produce high molecular weight PLLA. However, the use of solvent increases the difficulty of process control and polymer purification. Low molecular weight poly(lactic acid) may refer to polymers of lactic acid having a molecular

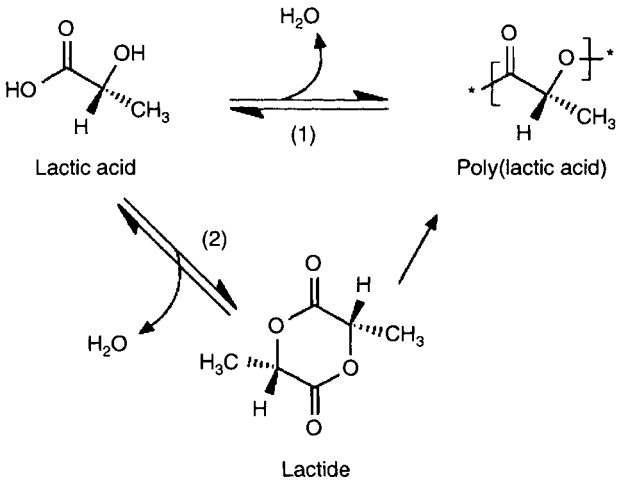

Figure 7.3 Direct and dimer routes to PLA [12].

weight of 3000–5000. Some of the catalysts commonly used in the synthesis of low molecular weight PLA are Sn (II) octoate, $SnCl_2$, diethyl zinc, 4-(dimethyl-amino) pyridine (DMAP).

7.3.1.1 Polycondensation of Lactic Acid

In general, the polycondensation of lactic acid proceeds stepwise in a similar manner to the esterification of a diacid with a diol. The molecular weight of the resulting polymer increases in proportion to the reaction time. During the polycondensation, an amount of water is produced as a by-product, which hydrolyzes the resulting polymer and thus, decreases the molecular weight of the polymer. Therefore, it is very important to choose a method that removes the water formed effectively from the reaction system. A variety of dehydration techniques are known which include: (1) increasing the stirring rate during the reaction, (2) using much reduced pressure and (3) introducing nitrogen gas flow. However, these techniques have fundamental limitations, because the viscosity of the reaction system increases with the increase in the molecular weight of the resulting polymer. The poly(lactic acid) is in a star shape which shows a lower melting viscosity, thereby solving the problem of efficiently removing the water formed during the reaction.

In principle, two processes are available for preparing a star form of poly(lactic acid), namely, a direct polymerization of lactic acid using a multifunctional reagent, and a two-step process comprised of preparing a straight chain poly(lactic acid) followed by coupling. Between both of these, the former process is preferred to produce the star-shaped poly(lactic acid). The reaction mechanism for forming the star-like molecular structure of the invention depends on the difference in the reactivity of the polyhydroxyl compounds and lactic acid used in the reaction. In the initial stage of the reaction, all primary hydroxyl groups of polyhydroxyl compounds are first

reacted with lactic acid to form a small star-shaped structure. The molecular chains grow while maintaining their star-shaped structure by the subsequent reaction of lactic acid, resulting in the desired polymer in a star form having a high molecular weight of above 30,000. In the latter process, the polycondensation can be carried out by heating the poly(lactic acid) having a low molecular weight (2,000–4,000) under reduced pressure using a conventional catalyst (antimony oxide) to get a high molecular weight PLA. The resulting polymer is colorless and nearly white. Such a high molecular weight range of the polymer is a distinctive feature in view of the fact that the poly(lactic acid)s obtained by the conventional polycondensation techniques show a lower molecular weight, for example, less than 10,000. Sudies have been done on how to obtain high molar mass polymer by manipulating the equilibrium between lactic acid, water and polylactic acid in an organic solvent [11]. In other studies a multifunctional branching agent was used to produce star-shaped polymers [12]. In the presence of bifunctional agents (dipoles and diacids) they form telechelic polymers, which can be further linked to produce high molar mass polymers using linking agents such as diisocyanate [13].

7.3.1.2 Ring-Opening Polymerization of Lactic Acid

The ring-opening polymerization route includes polycondensation of lactic acid followed by a depolymerization into the dehydrated cyclic dimmer (lactide), which can be polymerized via ring-opening to get high molar weight polymers. The depolymerization is conventionally carried out by increasing the polycondensation temperature and lowering the pressure, and distilling off the produced lactide. The various methods of ring-opening polymerization such as solution polymerization, bulk polymerization, melt polymerization and suspension polymerization are used [14]. Depending on the initiator/co-initiator system, the ring-opening polymerizations can be carried out via a coordination/insertion, anionic, cationic, zwitterionic, active hydrogen, or free-radical mechanism. It is catalyzed by compounds of transition metals such as tin, aluminium, lead, zinc, bismuth, iron and yttrium [14]. Other ring formed monomers can also be incorporated into the lactic acid based polymer by ring-opening copolymerization. The advantage of ring-opening polymerization is that the chemistry of the reaction can be accurately controlled, thus varying the properties of the resultant polymer in a more controlled manner. Various authors have studied the synthesis of different molecular weight polymers. It has been reported that the high molecular weight of poly(lactic acid) can be synthesized by one step polycondensation if appropriate azeotropic solvents are employed. The catalyst concentration, polymerization time and temperature cause profound effects on the polymer yield, molecular weight and optical rotation. The synthesis of poly(lactic acid) through polycondensation of the lactic acid monomer gave weight average molecular weights lower than 1.6×10^4, whereas ring-opening polymerization of lactides gave average molecular weights ranging from $2 \times 10^4 - 6.8 \times 10^5$ [15]. The monomer conversion

and average molecular weights show a maximum at a catalyst concentration of stannous octoate of 0.05%.

The mechanism for the polymerization of lactide can be cationic, anionic, coordination or free radical polymerization (Auras, Rafael) as discussed below:

Cationic polymerization takes place in the presence of any of the following catalyst: (a) protic acid such as HBr, HCl, triflicacid, etc., (b) lewis acid such as $ZnCl_2$, $AlCl_3$, etc., and (c) alkilating or acylating agents such as $Et_3O^+BF_4$, etc., as shown in Figure 7.4 [12].

Anionic polymerization proceeds by nucleophilic reaction of the anion with the carbonyl and the subsequent acyl-oxygen cleavage. This reaction produces an alkoxide end group, which continuously propagate to prepare polylactic acid (Figure 7.5) [12].

Coordination/Insertion polymerization employed less reactive metal carboxylates, oxides and alkoxides. Polymerization takes place in the presence of tin, zinc, aluminum and other heavy metal catalysts with tin (II) and zinc yielding the purest polymers. The mechanism is shown in Figure 7.6 [12].

A degradable high molecular weight poly(lactic acid) can be produced by the following mechanism: (1) Having a terminal end group of one of the

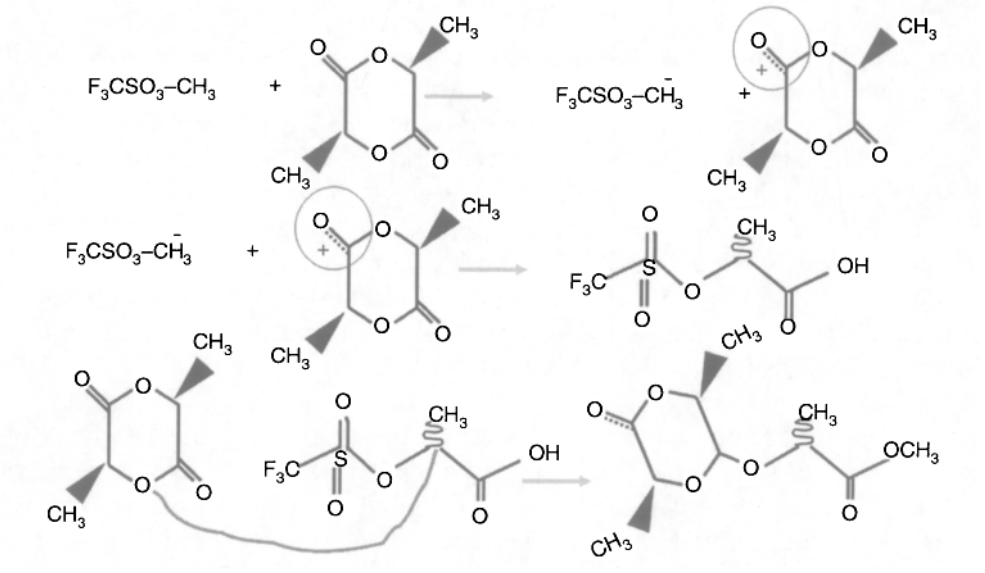

Figure 7.4 Cationic polymerization of lactide [12].

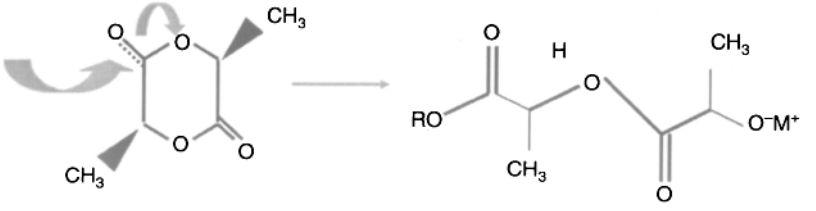

Figure 7.5 Anionic polymerization of lactide [12].

carboxyl or hydroxyl groups with low molecular weight poly(lactic acid) units coupled with linking agents of diisocyanates, bis-epoxides, bis-oxazolines and bis-ortho esters, (2) using azeotropic dehydration condensation of lactic acid (continuous removal of water) and, (3) ring-opening polymerization of lactide as shown in Figure 7.7. The resulting high molecular weight poly(lactic acid) can be used for applications taking advantage of the improved physical properties. Cargill Dow LLC and Mitsui Chemicals are the main producers of the poly(lactic acid). The complete process used by Cargill Dow LLC to prepare the poly(lactic acid) is shown in Figure 7.8 [16]. This process involves the synthesis of lactic acid by fermentation (using corn as a carbon source), purification and polymerization of lactic acid.

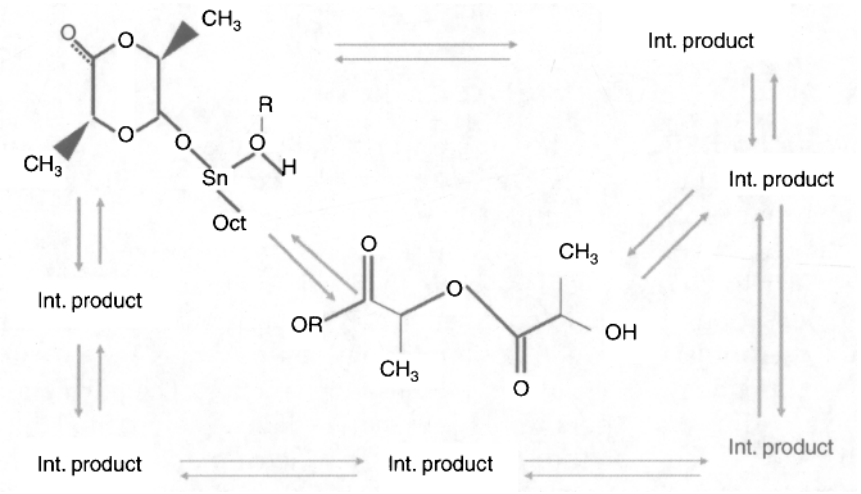

Figure 7.6 Co-ordination/insertion polymerization of lactide [12].

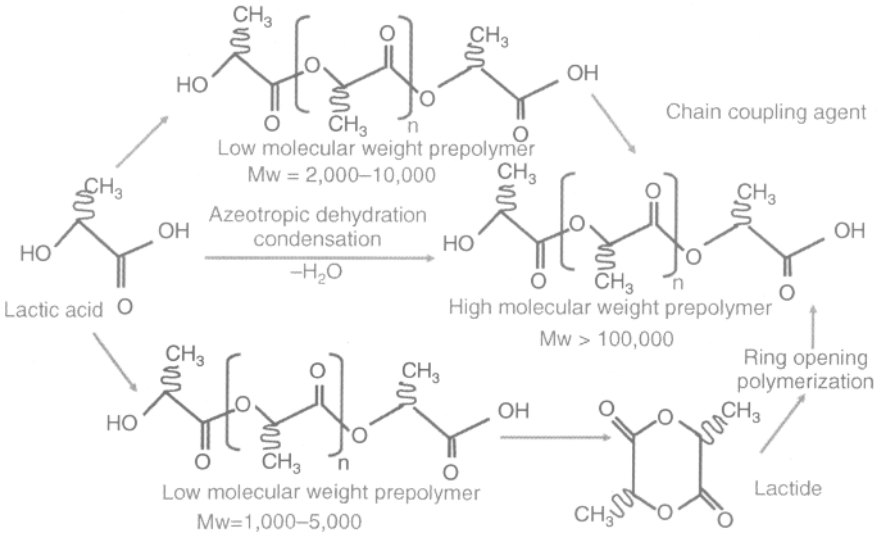

Figure 7.7 Methods for high molecular weight PLA [17].

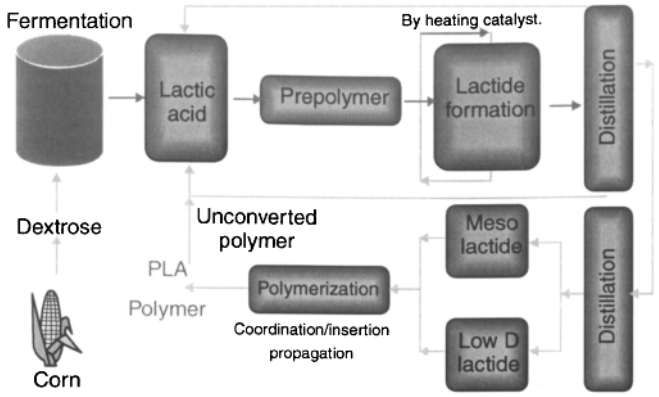

Figure 7.8 Synthesis of polylactic acid using renewable carbon source [16].

7.3.2 Polymerization of Glycolic Acid

Poly(glycolic acid) (PGA) is the simplest linear aliphatic polyester. Both PGA and the copolymer poly(glycolic acid - co - lactic acid) (PGA/PL) are used as degradable and absorbable sutures.

Glycolic acid can be catalytically dimerized into glycolide, which can then be polymerized using a ring-opening polymerization. Another simple process is to use a condensation reaction to polymerize glycolic acid; however, this process yields a lower molecular weight polymer than the ring-opening polymerization due to the side products of the condensation reaction. The polycondensed monomers create weak spots in the polymeric chains, thereby making them susceptible to degradation through hydrolysis. The ring-opening polymerization is a multi-step process that begins with the low molecular weight glycolic acid polymer. After the low molecular weight polymer is obtained, the glycolide is distilled by heating at low pressure. Although there are a variety of catalysts available such as antimony compounds, zinc compounds, and alkoxides, stannous octoate is preferred because it is approved by the US Food and Drug Administration (FDA) as a food stabilizer [17].

The ring-polymerization is started with the initiator and glycolide at a temperature of 195°C. After two hours at this temperature, the temperature is increased to 230°C. After the polymer solidifies, the high molecular weight polymer is obtained [18]. Another way to prepare the polymer is to induce a solid-state polycondensation of halogenoacetates. After heating the halogenoacetate between 160 and 180°C under nitrogen, polyglycolide is formed. The side-product salts can be easily removed by washing the polymer with water [19]. Polyglycolide is shown in Figure 7.9.

Polyglycolide has superior properties due to its greater stereoregularity. It has crystallinity between 45 and 55%, a glass transition temperature between 35 and 40°C, and a melting temperature between 225 and 230° C. It also has a higher strength and modulus of 7 GPa [20]. Polyglycolide is degraded by random hydrolysis into glycolic acid. According to Middleton, there is a two-step process: First, water diffuses into the amorphous regions and cleaves the ester bonds there. After these regions are eroded, the crystalline regions are also

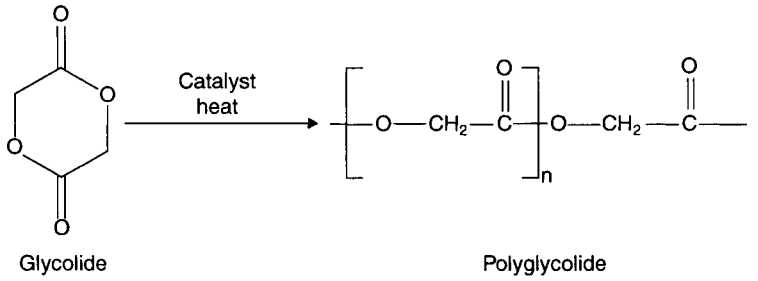

Figure 7.9 Polymerization of glycolide to poly glycolide.

dissolved [20]. Relative to PLA, PGA dissolves much quicker. In studies with medical sutures, PGA has been shown to lose 50% of its strength in only two weeks and all of it after only a month [21]. PGA is produced by Dupont as an aliphatic–aromatic copolymer, either under the trade name Biomax® or in form of aramid fibers with the name Kevlar®. It combines the excellent material properties of aromatic PET (polyethylene terephthalate), and the biodegradability of aliphatic polyesters. Polyglycolide presents many problems with processing due to its high melting temperature of 220°C. Although it can still be meltspun at greater temperatures (around 250°C), this creates a big problem in drug delivery because most drugs are decomposed or degraded at temperatures less than 220°C [22]. To overcome this, blends and copolymers are being investigated. These polymers vary in biodegradation time, and hence copolymers can be produced with a wide-variety of degradation times, depending on the use. The biodegradation time can be varied by changing the percent composition of the copolymer.

7.3.2.1 Poly (lactic-co-glycolic acid) (PLGA)

Many natural biopolymers that we see include cellulose, starch, and chitin, while the more common synthetic polymers consist of poly(lactic acid) (PLA), poly(-caprolactone) (PCL), poly(glycolic acid) (PGA) and poly(lactic-co-glycolic acid) (PLGA) [17]. The sutures used most often are multifilament, with good handling characteristics. The most popular and commercially available are the sutures made from PGA, PLA and their copolymers. For laying continuous sutures, however, braided sutures with non-smooth surfaces are not useful. In such cases only monofilament sutures with smooth surfaces are useful, because PGA or PLA proved to be too stiff and inflexible. The more flexible polydioxanones and polyglyconates can be used as sutures due to their low bending moduli [23, 24]. The copolymer, poly(lactic-co-glycolic acid) (PLGA), can be produced using the same techniques, as seen in Figure 7.10.

PLGA copolymers have a glass transition temperature between 40 and 60°C, their melting point and percent crystallinity depend on the percent composition. For example, 5:95 ratio of PGA to PLA copolymer has a melting temperature of 173°C while 9:1 ratio of PGA to PLA copolymer has a melting point of 201° C [25]. The most common copolymer of PGA is the 50/50 copolymer with lactic acid, PLGA. Currently, PLGA is mostly produced for use in drug

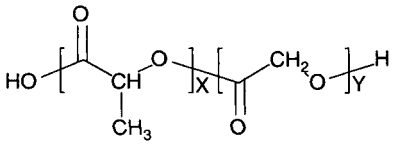

Figure 7.10 Copolymer of lactic- and glycolic acid.

delivery systems. To do so, PLGA and the drug are dissolved in a solvent, such as dichloromethane (DCM). Upon solvent evaporation, a microparticle of the PLGA forms and it is further cast to obtain drug-loaded polymer films to be used in drug delivery systems [26].

7.3.2.2 Other Polymers of PGA

Dexon is made of poly(glycolic acid), the first synthetic polymer developed especially for producing surgical thread. The fibers of the yarn obtained are precisely woven into a high-flexibility thread, very easily handled and with high knot security. This material undergoes hydrolytic decomposition in humans, causing minimal tissue reaction. The minimum absorbing period was observed 15 days after implantation, complete absorption took place within 60–90 days.

Polygalactin 910 is a copolymer of glycolide and lactide, obtained from glycolic and lactic acid in 9:1 ratio. The multi-fiber threads, called Victyl or Polisorb, are coated, transparent, or dyed purple. For Vicryl Rapid threads a material with smaller relative molecular mass is used, and as a result is absorbed more rapidly.

Mexon is a synthetic single-fiber thread with slow absorption characteristics, made of a copolymer of glycolic acid and trimethyl carbonate. Three weeks after implantation it retains about 55% of its initial resistance; complete absorption takes place after 26–30 weeks. The products of hydrolytic thread decomposition are: carbon dioxide, -hydroxybutyric acid, and glycolic acid.

Monocryl (Poliglecaprone 25) is a glycolide and -caprolactone copolymer. The thread is nontoxic, but causes a delicate reaction during absorption, which takes place *in vivo* by way of hydrolysis.

7.4 Applications

Biodegradable polymers are used in an increasingly large number of mass produced applications such as packaging, paper coating, fibers, films, and other disposable articles, as well as in biomedical applications. Lactic acid-based biodegradable polymers have medical applications as sutures, orthopedic implants, and in controlled drug release systems, etc. Polymers of lactic acids are biodegradable thermoplastics. These polymers are transparent and their

degradation can be controlled by adjusting the composition, and the molecular weight. Their properties approach those of petroleum derived plastics. Poly L-lactic acid with a low degree of polymerization can help in controlled release or degradable mulch films for large-scale agricultural applications [27]. PLGA degrades by hydrolysis of its ester linkages in the presence of water. It has been shown that the time required for degradation of PLGA is related to the monomers' ratio used in production: the higher the content of glycolide units, the lower the time required for degradation. An exception to this rule is the copolymer with 50:50 monomers' ratio which exhibits the faster degradation (about two months). In addition, polymers that are end-capped with esters (as opposed to the free carboxylic acid) demonstrate longer degradation half-lives. Because of its relative high price, the actual applications of PGA and PLGA are only seen in the biomedical sector. Already used in Lupron Depot for the treatment of prostate cancer, PGLA's use in modern plastics is only hindered by its price [28]. With PLA at $1.30 per pound and PGA around $1.50 per pound [28] PLGA's price hovers in the middle, approximately correlating to the percent composition [20].

7.5 Conclusions

PLA, PGA and their copolymers have the greatest potential market because they are compostable and biodegradable thermoplastics. The monomers (lactic acid and glycolic acid) can be derived from annually renewable agricultural resources. New technologies for mass production of these polymers promise to lower its cost and widen its applications in packaging, including food packing and bottles. To lower its price and increase its use as a common plastic, the monomers of lactic acid and glycolic acid need to be produced in greater amounts. Since the world continues to move towards renewable resources for most materials, wide-spread use of plastics such as PLA, PGA, or PLGA will grow along with it.

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8

Characteristics and Applications of Poly(lactide)

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Abstract
Poly(lactic acid) (PLA), is one of the biopolymers already available in large quantities produced by industrial fabrication in a number of different commercial grades. Its promising properties make it suitable for different types of applications in the biomedical and technical field. This chapter will focus on PLA properties which are mainly used for applications in high volume markets, such as technical applications or packaging. The main focus of this chapter will be the production of lactic acid, the synthesis, the degradation and biodegradation of PLA. Knowledge of the relationship between the process and the polymer properties allows for the tailoring of the final material for a given application. The main fields of technical applications of PLA are reviewed, and their main advantages and limitations are pointed out.

Keywords: Poly(lactic acid), poly(lactide), biopolymer, PLA synthesis, degradation, crystallization, mechanical properties, barrier properties, process, applications, automotive, packaging

8.1 Introduction

Poly(lactic acid) or poly(lactide) (PLA) was first reported on in 1932 by Wallace Carothers and his co-workers, chemists at DuPont, whose aim was to obtain high molecular weight polymers. Their work showed the dimerization of polycondensated lactic acid into lactide and the ring-opening polymerization of lactide [1]. Further work by DuPont, patented in 1954, yielded a high-molecular weight product due to improved lactide purification processes [2]. However, the aliphatic polyester PLA was abandoned because it was considered not sufficiently stable at the time due to its susceptibility to hydrolytic degradation. It was only in the 1960's that interest was renewed because of the advantages of hydrolysable structures perceived to be useful for biomedical applications. In 1972, high-strength, biocompatible fibres for medical resorbable sutures were introduced by Ethicon. Since the 1970's the biomedical applications of PLA are fields of active research and have brought about a number of major developments in controlled drug release and implants. However, the high cost of lactic

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (183–224)

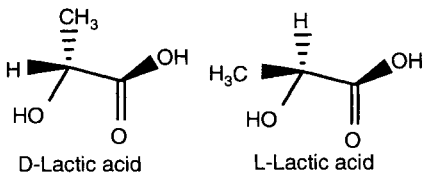
acid, and therefore of the resulting polymers, restricted commodity applications. In the late 1980's, advances in the fermentation technique for obtaining lactic acid enabled a substantial increase in world-wide production and a substantial decrease in cost. In 1992, Cargill Inc. patented a solvent-free process and novel distillation technique to convert lactic acid into high-molecular weight polymers based on the ring-opening polymerization (ROP) method. The first industrial production plant of PLA started production in 2002 and has actually an estimated production potential of 140 ktonnes per year. Several other investments in industrial scale production facilities are underway, which has resulted in PLA being the second most produced bioplastic at present time after starch-based materials. PLA is already successfully applied in the biomedical field, and a recent publication has also shown its substitution potential for commodity applications, such as in textiles or packaging [3].

8.2 Production of PLA

8.2.1 Production of Lactic Acid

The basic constitutional unit of PLA is lactic acid. Lactic acid (2-hydroxy propionic acid) is an a-hydroxy acid with an asymmetric carbon atom and exists either as L(+) or D(-) stereoisomer, as shown in Figure 8.1.

The L- isomer is produced in humans and other mammals, whereas both the D- and L-enantiomers are produced in bacterial systems. Lactic acid can also be derived chemically from renewable resources such as ethanol or acetaldehyde or from chemicals coming from coal (e.g. acetylene) or oil (e.g. ethylene) [4]. However, the large majority of lactic acid produced today is obtained by bacterial fermentation of simple sugars, using homofermentative strains of the genus *Lactobacilli*. The homofermentative pathway yields 1.8 moles of lactic acid per mole of hexose, and conversion rates of glucose are higher than 90 % [5]. Processing conditions are generally batch fermentation at pH of 5.4–6.4, temperature around 40°C and low oxygen concentration. The lactic acid produced is neutralized with $Ca(OH)_2$ or $CaCO_3$. Strains such as *L. amylophilus, L. bavaricus, L. casei, L. maltaromicus,* and *L. salivarius* produce L-lactic acid exclusively, while strains such as *L. delbrueckii, L. jensenii,* and *L. acidophilus* yield mixtures of both L- and D-lactic acid. The main carbon sources are glucose and maltose from corn, sugar beet, sugar cane or potato, supplemented with other complex nutrients, supplied by complex sources, such as corn steep



Figur 8.1 Stereoisomers of 2-hydroxypropionic acid (lactic acid).

liquor or yeast extract [6]. Down-stream processing includes filtration of the culture broth to remove insolubles and biomass, evaporation, recrystallization, and acidification of the calcium lactate by sulphuric acid to yield crude lactic acid. The insoluble calcium sulphate is discarded, which generates one metric tonne of waste ($CaSO_4$ called gypsum) per metric tonne of lactic acid. For use of lactic acid in food and pharmaceutical applications, further purification by distillation is required [7].

Main bottlenecks of the process are the inhibition of bacterial growth at a pH lower than 4, while the pKa of lactic acid is 3.78 causing the need for continuous neutralization of the culture medium. Also, the cost of pre-treatment of raw materials is high because of the need for saccharification to obtain simple sugars, and the cost and waste generation during downstream processing has to be considered as well. To address these points, optimization of production strains and downstream processing is required.

Recombinant strategies have been employed to generate more pH tolerant lactic acid bacteria and to enable the use of complex carbon sources, such as starch or cellulose, or to generate lactic acid producing strains of other microorganisms, such as *Escherichia coli*, *Corynebacterium glutamicum* or yeasts. Okano *et al.* [8] recently published a review about different strategies for strain optimization, showing for example a result from their group in which they redirected metabolic pathways in *L. plantarum ΔldhL1* using arabinose coming from hemicellulose in order to obtain efficient production of lactic acid and significantly decrease the by-production of acetic acid. The use of hemicellulose for the fermentative production of chemical building blocks is one of the major challenges for the utilisation of lignocellulosic feedstocks, because pentoses are unavailable to most microorganisms. The use of *E. coli* and *C. glutamicum* seems to be promising. *E. coli* is, for example, able to grow on simple mineral salt media without the need for expensive nutriments such as vitamin B. However pH tolerance of those bacteria is very low and lactic acid yield needs to be improved [8].

A complementary strategy to increase the efficiency of the lactic acid production is process intensification. Continuous removal of lactic acid or lactate is required, which can be achieved by adsorption, extraction, and membrane separation. Membrane separation has been the object of intensive research in recent years, because it cumulates the advantages of not requiring process steps for regeneration and recycling, and offers the possibility of cell recycling into the bioreactor to maintain high cell densities. The primary techniques are microfiltration, ultrafiltration, nanofiltration, reverse osmosis and electrodialysis. One of the main challenges is membrane fouling by microbial cells and proteins. Furthermore, a selective separation of lactic acid is desirable, which also allows for the recycling of unused nutrients into the bioreactor. However, the retention of neutral solutes, such as glucose, by nanofiltration or reverse osmosis in the presence of charged ones generally decreases, probably because of increased membrane charge density in the presence of charged solutes [9]. To overcome these drawbacks, multi-stage systems are probably required. One proposal is a two-step process starting with microfiltration which is less

susceptible to fouling, and following it with nanofiltration, which is capable of separating lactic acid in the permeate stream and while retaining useful solutes for recycling into the bioreactor [9].

8.2.2 Synthesis of PLA

Lactic acid based polymers can be synthesized by different routes, as shown in Figure 8.2. Globally, there are two routes to obtain high molecular weight polymers: Direct lactic acid condensation, often including the use of coupling agents to increase molecular weight and ring-opening polymerization (ROP) of lactide [10]. The nomenclature used in literature for these polymers is sometimes contradictory. Polymers derived from direct polycondensation should be referred to as poly(lactic acid) and polymers synthesized by ROP of lactide as poly(lactide). Both types are generally named PLA. In the function of stereochemistry of the polymer chain, different types are obtained and summarized in Table 8.1.

PLA polymerization by direct polycondensation (route 1 in Figure 8.2), the least expensive route, yields low molecular mass polymers [11], having mechanical properties which are insufficient for most applications. To increase molecular weight, chain coupling agents, such as anhydrides, epoxides or isocyanates, are added. They react preferentially with the hydroxyl or the carboxyl group leading to different reaction rates. An answer to this problem is the polycondensation of lactic acid in the presence of diacids or diols, being difunctional monomers, which leads to the formation of telechelic prepolymers, being either all hydroxyl or all carboxyl terminated [12]. However, a purification step has to be performed to remove unreacted coupling agents

Table 8.1 Stereoisomers of PLA.

PLA form	Structure
Isotactic poly(L-lactide), PLLA	LLLLLLL
Isotactic poly(D-lactide), PDLA	DDDDDDDD
Random optical copolymers	Random level of meso or D-lactide in L-lactide or D-lactic acid in L-lactic acid
Stereocomplex PLLA/PDLA	LLLLLLL mixed with DDDDDDDD
PLLA/PDLA stereoblock complexes	LLLLLLLLDDDDDDDD
Syndiotactic poly(meso-lactide)	DLDLDLDL Al-centered R-chiral catalyst
Heterotactic poly(meso-lactide)	LLDDLLDDLLDD
Atactic poly(meso-lactide)	No stereocontrol
Atactic poly(lactide), PDLLA	No stereocontrol

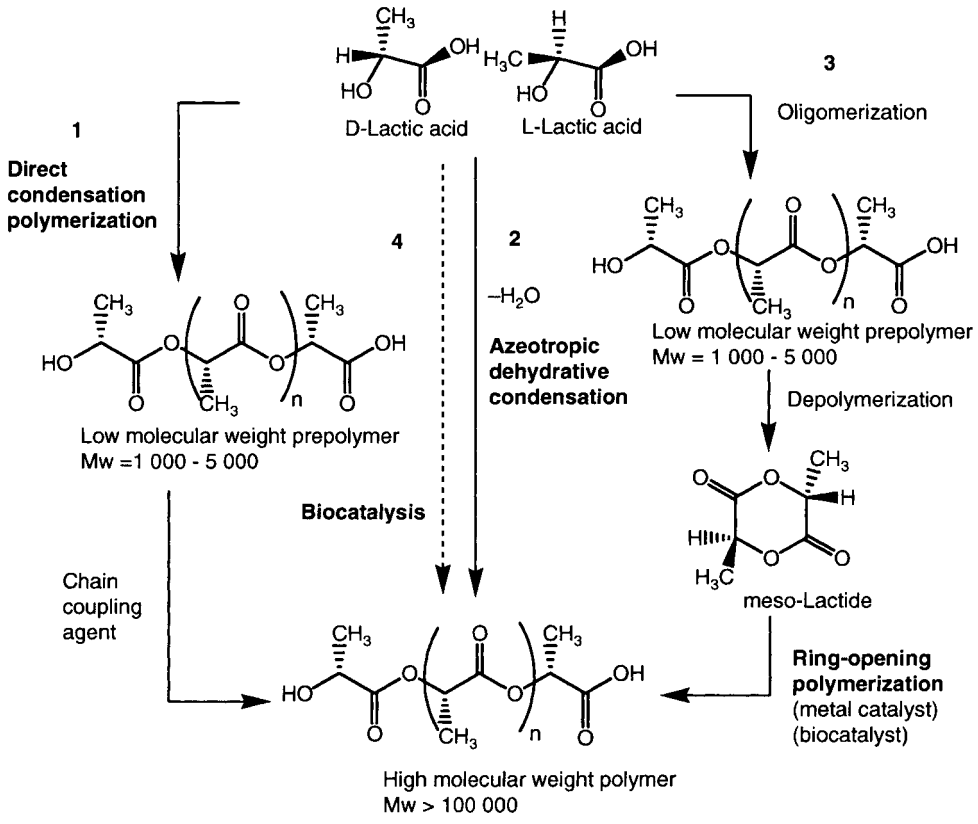


Figure 8.2 Main routes for the synthesis of PLA. Adapted from [5].

and prepolymers [10]. The multiplication of the steps for this polymerization induces an enhancement of the production cost.

Azeotropic dehydration and condensation polymerization (route 2 in Figure 8.2) yields directly high molar mass polymers. The procedure, patented by Mitsui Toatsu Chemicals [13, 14], consists of the removal of condensation water via a reduced pressure distillation of lactic acid for 2-3h at 130°C. The catalyst (in high amounts) and diphenyl ester are added and the mixture is heated up to reflux for 30–40 h at 103°C. Polycondensated PLA is purified to reduce residual catalyst content to the ppm range [5, 10, 15].

The ROP of lactide (route 3 in Figure 8.2) is nowadays by far the route that is most employed to obtain high molecular weight polymers. This route requires several steps. First, a direct polycondensation reaction is carried out to produce a low molar mass prepolymer [16]. The prepolymer is depolymerized in order to obtain lactides (3,6-dimethyl-1,4-dioxane-2,5-dione), which are dehydrated cyclic dimers, which are the starting material for ROP into high molar mass polymers. Depolymerization is done under reduced pressure, high temperature and distillation of the lactide product. Three forms of lactide: L,L-lactide, D,D-lactide and D,L-lactide (meso-lactide) (Figure 8.3) are obtained as a function of the stereochemistry of the feedstock, temperature and catalyst [10].

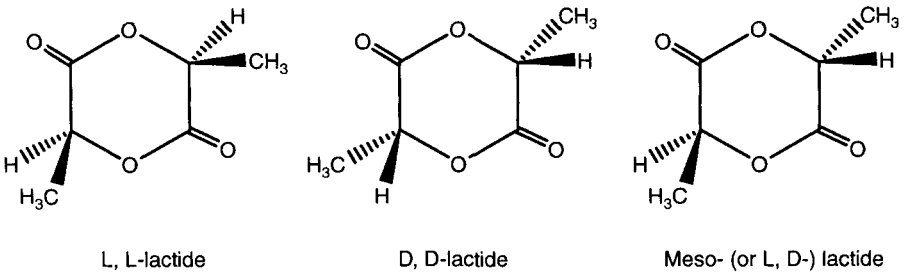


Figure 8.3 Stereoforms of lactide (3,6-dimethyl-1,4-dioxane-2,5-dione).

Polymerization through lactide formation is currently used by Cargill (NatureWorks™), which patented a continuous process [17, 18].

ROP is carried out in solution, in the melt, in the bulk or in suspension. The involved mechanism can be ionic (anionic or cationic), coordination-insertion or free-radical polymerization [19].The cationic polymerization is initiated by only two catalysts, trifluoromethane-sulphonic acid and its methyl ester [10, 15]. Initiators such as potassium methoxide, potassium benzoate, zinc stearate, *n-*, *sec-*, *ter*-butyl lithium or 18-crown-6-ether complexes are added for the anionic polymerization to induce a nucleophilic reaction on the carbonyl to lead to an acyl-oxygen link cleavage. According to Jedkinski *et al.* only the primary alkoxides, such as the first mentioned catalyst, can yield polymers with negligible racemization, transesterification and termination [10].

For a large scale production, the melt and bulk polymerization is preferable to the solution process. Tin [20], zinc, iron [21], aluminium [22] or other heavy metal catalysts with tin(II) and zinc are used to produce the high molecular mass polymers. The best results are obtained with tin oxide and octoate at 120–150°C with conversions above 90% [10]. Stannous octoate (or Stannous-2-ethyl hexanoate) is an efficient catalyst for PLA, FDA approved due to its low toxicity, and capable of inducing the production of high mass polymers with low racemisation [10]. However, the reaction mechanism is still being debated. Two mechanisms are proposed to explain the reaction. The first one postulates that the reaction is primed by monomer or impurities and catalysed by a Lewis acid, stannous octoate [23, 24]. The second one corresponds to the mechanism of coordination-insertion which takes place in two steps: (i) covalent bonds of a complex formed from a monomer and an initiator rearrange, (ii) a second monomer reacts with this intermediate, which makes possible the ring-opening by the cleavage of the oxygen-acyl bond [15, 20, 25].

Reaction time, temperature, ratio monomer/catalyst and catalyst type, chosen for the polymerization, are limiting parameters for yield, degradation and polymerization products.

In the case of biomedical applications, metallic impurities coming from the organo-metallic catalysts of ROP may be of concern, because they can accumulate within matrix remnants after degradation [26]. A recent strategy to reduce such problems and to shift polymer synthesis further towards green chemistry is the use of enzymes as catalysts. Kobayashi and Makino [27] published a

recent general review on the topic and Albertsson and Srivastava [28] focused more precisely on ROP. In most cases, as in the case of PLA, enzymes used are lipases. Lipases are the most versatile class of biocatalysts for the synthesis of organic compounds, because they can work with a wide variety of substrates, and are stable in organic solvents and at elevated temperatures. Being part of the hydrolase family, lipases catalyze the hydrolysis of fatty acid esters. In organic solvent, they can be used for the catalysis of esterification and transesterification reactions. Matsumura *et al.* [29] reported the lipase PS catalyzed ROP of lactide in the bulk yielding molecular weights (M_w) up to 1.26×10^5 $g.mol^{-1}$. Meso-lactide gave higher molecular weight than L,L-lactide or D,D-lactide. Recently, Novozyme 435 was used as a catalyst in toluene solution. The enantioselectivity of the enzyme enabled the selective polymerization of D,D-lactide, but no polymerization of L,L-lactide or meso-lactide occurred. However, molecular weight was quite low (M_n = 3 300 $g.mol^{-1}$) [30]. Novozyme 435 seems to catalyze the ROP of L,L-lactide in ionic liquids. In [HMM] PF_6] at 90°C, yields up to 65% could be obtained, but still molecular weight remained low (max. $M_n = 3.78 \times 10^4$ $g.mol^{-1}$) [31]. Generally speaking, the use of lipase in ROP still suffers from low yields and/or low molecular weights, but the problems are the subject of an actual research effort.

Whole-cell synthesis of lactate containing polyesters is a new route (route 4, Figure 8.2) of research that's still in the beginning stages. Taguchi *et al.* reported in 2008 [32] the discovery of a lactate-polymerizing enzyme, being a PHA synthase with acquired polymerizing activity of the lactide in lactide-CoA. $PhaC1_{PS}STQK$ is an engineered class II PHA synthase from *Pseudomonas sp.* and is able to form a co-polymer P(LA-*co*-3HB) inside engineered *Escherichia coli*. Polymer cell content in *E. coli* amounted to 51% of the dry mass and LA molar fraction in the polymer was 28%. At this stage, yields and lactide fraction appear moderate, however, the groundwork has been laid for future mutation/selection or modified strategies of selection in similar enzyme lines for generating a lactide enriched polymer, and eventually a PLA homopolymer.

Reactive extrusion is a continuous process for polymerizing the lactic acid by ROP in the molten state in one single process step. However, this method requires a fast reaction rate which is determined by the residence time in the extruder (typically 7 min). The extruder presents a sophisticated design of screw which makes it possible to assure the blending and the shearing in the molten state. A common design is an intermeshing co-rotating twin-screw extrusion [33]. The short reaction times require efficient catalysts with reduced side-reactions such as inter- and intramolecular transesterification due to the high reaction temperatures. To limit these reactions a Lewis acid, triphenylphosphine in an equimolar complex with $Sn(Oct)_2$, was used to enhance the polymerisation rate and delay the occurrence of undesirable back-biting reactions for a monomer-catalyst molar ratio of at least 5000 [34, 35]. Jacobsen *et al.* showed that the number average molecular weight, around 80 000 $g.mol^{-1}$, the polydispersity and the degree of conversion vary according to the mass flow rate and the screw speed [35].

Recently stereocomplexes of PLA appeared on the market and lead to promising applications in durable devices (see below: PLA applications). The stereocomplexes are defined as the association of polymers with different tacticity or conformation. Three synthesis routes are used to produce a PLA stereocomplex, either in solution or in melt state during polymerization or hydrolysis. The complexe formation is possible with (i) two monomers (L-lactide and D-lactide), (ii) polymer and monomer or (iii) two polymers (PLLA and PDLA). This synthesis is often performed with stannous tin and 1-dodecanol (lauryl alcohol) as initiator or co-initiator of the reaction [36-38]. According to Tsuji *et al.* [38], some other parameters affect the formation of stereocomplexes:

- Equimolarity of the blending of D-lactide and L-lactide units.
- Low molecular weight for both the isomeric polymers.
- Sufficiently long sequences of both isotactic L-lactide and D-lactide units.

Moreover, the solvent and the blending mode are also parameters which strongly influence the stereocomplexation [36].

8.3 Physical PLA Properties

PLA properties are strongly dependent on their molecular weight [10] and stereochemistry, being L- and D-lactic acid content [39]. Indeed a PLLA or PDLA homopolymer can develop a crystalline structure whereas an atactic polymer whose L-lactic acid content is below 93% remains amorphous. Consequently, the polymer structure, crystalline or amorphous form, can be at the origin of modification in the thermal, optical, physical, mechanical, and barrier properties of PLA.

Very few values of the density of PLA are shown in literature. Auras *et al.* reported that the density of amorphous PLLA is 1.248 kg/L whereas it is 1.290 kg/L for the crystalline PLLA [5, 15, 40]. NatureWorks LLC gives, according to the polymer type and the L-lactic acid content, an density value between 1.24 and 1.25 kg/L for its amorphous PLA grades [41]. In agreement with these values, Auras *et al.* measured 1.240 ± 0.002 kg/L for poly(98% L-lactide) and 1.243 ± 0.002 kg/L for poly(94% L-lactide) [5].

The solubility of PLA is dependent on the molar weight and the crystallinity degree of the polymer. For the enantiopure PLA, chloroform and other chlorinated organic solvents, such as furan, dioxane, dioxolane and pyridine are good solvents. In addition to these organic solvents, the non-enantiopure PLA is soluble in ethyl acetate, dimethylsulfoxide, tetrahydrofuran, acetone, xylene, methyl ethyl ketone, ethyl lactate and dimethylformamide. However, lactic acid based polymers are not soluble in water, alcohol (e.g. ethanol, methanol), isopropyl ether, or unsubstituted hydrocarbons (e.g. cyclohexane, heptane) [19].

The optical properties of PLA have been measured. Its refractive index is characteristic of its structure and is directly correlated to its isotropy. Tsuji *et al.* [42] measured the refractive index at 25 °C and PLA concentration of

1 g.L^{-1} in chloroform at 589 nm to -156° and 156° dm^{-1} g^{-1} cm^3 for PLLA and PDLA respectively. In the range of 190–800 nm, PLA has been compared to other standard commercial films. Experimental results are shown in Figure 8.4.

The infrared spectrum of commercial PLA was determined by Fourier Transform Infrared (FT-IR). Table 8.2, which contains data published in the

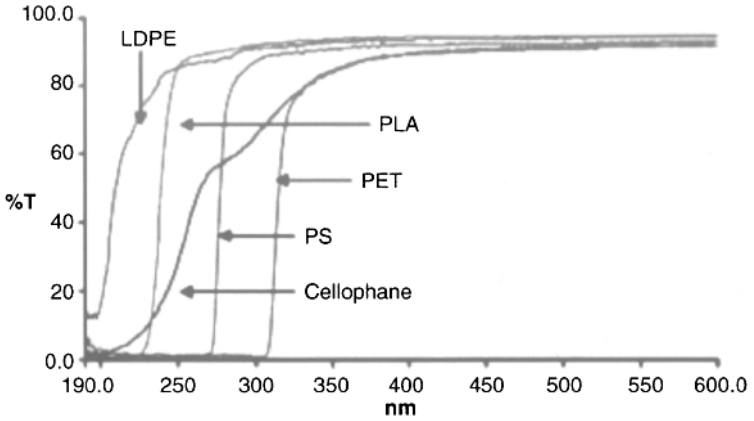


Figure 8.4 Transmission versus wavelength for LDPE, PLA(98% L-lactide), cellophane, PS and PET [5].

Table 8.2 Infrared spectroscopy peak band assignment for PLA.

Assignment	Peak Position (cm^{-1})	
	Furukawa et al. [43]	Auras et al.[5]
-OH stretch (free)		3 571
-CH- stretch		2 997 (asym), 2 946 (sym), 2 877
-C=O carbonyl stretch	1 752 (C), 1 744 (A)	1 748
-CH3 bend	1 450 (asym), 1 380 (sym), 1 356 (sym, C)	1 456
-CH- deformation (symmetric and asymmetric bend)	1 356, 1 265 (A)	1 382, 1 365
-C=O bend		1 225
-C-O- stretch	1 265, 1 210 (C), 1 179, 1 080	1 194, 1 130, 1 093
-OH bend		1047
-CH3 rocking modes	1 125	956, 921
-C-C- stretch	1 044	926, 868

C = Crystalline; A =Amorphous.

review paper of Auras *et al.* [5] and by Furukawa *et al.* [43], summarizes the main PLA absorption bands. Two bands are related to the crystalline and amorphous phase of PLA (98% L-lactic acid): One band at 871 cm^{-1} which is assigned to the amorphous phase, and one band at 756 cm^{-1} which is related to the crystalline phase [5]. As shown in Table 8.2, Furakawa *et al.* [43] showed that some other bands are linked to the crystallinity of PLA.

8.4 Microstructure and Thermal Properties

PLA is a semi-crystalline polyester. A typical DSC signal of PLLA is shown in Figure 8.5.

The crystallinity of PLA depends largely on its architecture, i.e. the stereochemistry of the monomers which constitute the polymer. PLA containing more than 93% of L-lactic acid is semi-crystalline in contrast to PLA with L-lactic acid between 50–93% which is amorphous. D-lactic acid, in high proportion, prevents PLA crystallization [5]. The crystalline phase proportion varies according to the L-lactic acid content. The higher the L-lactide percentage is, the more the chains can be organized and crystallize. So the PLLA crystallinity degree can reach 70% [45], whereas a PDLLA with a L-lactic acid content close to 93% can crystallize up to 45%.

8.4.1 Amorphous Phase of PLA

The glass transition temperature (Tg) of amorphous PLA lies between 55 and 60°C [5, 19, 46, 47] and is a function of the PLA molecular weight and

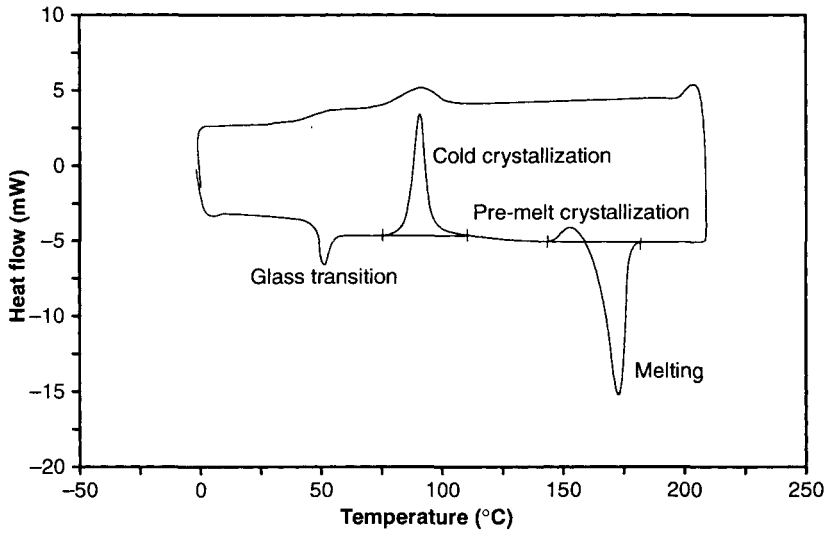


Figure 8.5 DSC trace on heating of neat PLLA displaying glass transition, cold crystallization, pre-melt crystallization, and melting. Adapted with permission from [44].

stereochemistry. An endothermic peak is usually superimposed on the glass transition. This enthalpic relaxation is due to the secondary molecular relaxation in the amorphous phase. It disappears when the sample is heated over the glass transition temperature [48, 49]. The Tg of semi-crystalline PLA shifts to higher temperatures compared to amorphous PLA, being between 60 and 80°C. The change in heat capacity of PLA at the glass transition (ΔC_p^0) varies according to author and the PLA grade. Arnoult *et al.* [50] measured 0.48 $J.g^{-1}.K^{-1}$ for a PDLLA with an estimated D -lactide content of 0.4% and Pyda *et al.* [51] measured 0.608 $J.g^{-1}.K^{-1}$ for a PDLLA with 1.5% of D-lactide.

The amorphous phase microstructure of PLA has been studied recently in an application of the three-phase model, stating that two phases can be distinguished in the amorphous phase, a mobile fraction (MAF) and a rigid fraction having a Tg shifted to higher values (RAF). Contrary to PET, the RAF in PLA is small. It increases however, once the maximum degree of crystallinity is reached (between 30 and 40% for PDLLA). Arnoult *et al.* showed that this increase is limited at 25% [50] of the sample mass whereas according to del Rio *et al.* the RAF fraction increases with the annealing time to reach almost 50% of the sample mass [52].

8.4.2 Crystalline Structure of PLA

The crystalline phase of poly(lactic acid) has been mainly studied in the case of PLLA, while only limited data is available on PDLLA or PDLA. Nevertheless, the homopolymers probably present a similar polymorphism, but their handedness of molecular chains in the crystal lattice is opposite [53]. PLLA crystallizes under three main forms dependent on the preparation conditions: α (α′, α″), β and γ.

The α-form is the most common form of PLLA. It expands under normal crystallization conditions, such as crystallization from molten, glassy state, or in solution [54]. The α-form is characterized by two antiparallel chains in a left-handed 10_3 helix conformation [54, 55]. As shown in Table 8.3, the chains are stacked in an orthorhombic or pseudo-orthorhombic crystalline unit cell. A notable structural feature of the α-form is the distortion of the helix conformation and the crystal structure change. According to the authors named in Table 8.3 the interactions between closest neighbour chains, that is to say the electrostatic dipole-dipole interactions, are the cause of these changing data.

The α′-form corresponds to a disordered α-form [65]. This crystal presents the same conformation of the α-form, but the stacking seems to differ. Cell unit dimensions are a = 1.074 nm, b = 0.620 nm and c = 2.880 nm. The ratio a/b corresponds to $3^{1/2}$, which is typical for a hexagonal crystal. The interlamellar space also changes with the crystalline form. It is higher in the case of α′-form than in the case of α-form. At temperatures lower than 100°C the α′-form is predominant, while between 100 and 120°C a mix of α and α′-form is observed [54, 55, 65-67]. The α′-form turns into a α–form upon a heating up to melting or upon an annealing at high temperature (120-160°C), meaning the unit cell becomes more compact [65, 68, 69].

Table 8.3 Previously reported data relative to the crystalline structure of the α-form of PLLA, adapted with permission from [56].

Authors	Crystal System	a (nm)	b (nm)	c (nm)	Chain Conformation
De Santis and Kovacs[57]	P-O	1.070	0.645	2.780	10_3 helix
Kalb and Pennings[58]	P-O	1.034	0.597		
Hoogsteen et al.[59]	P-O	1.060	0.610	2.880	distorted 10_3 helix
Kobayashi et al.[60]	O	1.050	0.610	2.880	10_3 helix
Brizzolara et al.[61]	O	1.060	0.605	2.880	distorted 10_3 helix
Miyata and Masuko[62]	O	1.078	0.604	2.870	
Iwata and Doi[63, 64]	O	1.070	0.615		
Sasaki and Asakura[64]	O	1.066	0.616	2.888	distorted 10_3 helix

P-O : Pseudo-Orthorhombic
O : Orthorhombic

The α''-form was discovered by Marubayashi *et al.* [70]. The α''-form is, like α'-form, a disordered α-form due to the exposition to high-pressure CO_2 below Tg. So below 40°C, for a pressure between 3 and 15 MPa, only the α''-form is obtained. This α''-form presents a poor chain packing and a lower crystal density, compared to α- and α'-form [70].

The β-form was detected the first time by Eling *et al.* in the beginning of the 80's upon hot-drawing the melt-spun or solution spun PLLA fibres to a high draw ratio [71]. Generally this form is obtained using high temperature and high draw ratio stretching [54]. The β-form is characterized by a chain number varying according to the author, with a 3_1 helical conformation. As shown in Table 8.4, the cell unit differs also: It is orthorhombic or trigonal [55, 72].

The γ-crystalline form, discovered by Lotz *et al.* has been the subject of only a few studies. It is obtained by epitaxial crystallization [53-55] on hexamethylbenzene (HMB) substrate, but the formation mechanism is still to be determined. The γ–form is characterized by two antiparallel helices with a 3_1 conformation packed in an orthorhombic cell unit whose dimensions are a = 0.995nm, b = 0.625nm, and c = 0.880nm [54].

8.4.3 Crystallization Kinetics of PLA

The PLA crystallization kinetics has been widely investigated, and most of the studies have been carried out on PLLA. The PLA crystallization, isothermal or non-isothermal, is related to the L-lactic acid content [39, 74, 75], the

Table 8.4 Previously reported data relative to the crystalline structure of the β-form of PLLA, adapted with permission from [56].

Authors	Crystal System	a (nm)	b (nm)	c (nm)	Chain Conformation
Hoogsteen et al.[59]	O	1.031	1.821	0.900	6 chains, 3_1 helix
Sawai et al.[73]	O	1.040	1.770	0.900	6 chains, 3_1 helix
Puiggali et al.[72]	T	1.052	1.052	0.880	3 chains, 3_1 helix

O : Orthorhombic
T: Trigonal

molar weight of the polymer [10] and the cooling or heating rate, and depends on the thermal history of the sample and the presence of nuclei in the matrix [76, 77]. In the appropriate conditions (crystallization time and temperature) [78, 79] the crystallinity degree of PLLA can reach 45–70%. The crystallinity degree is calculated according to the equation 8.1 in which ΔH_m is the measured enthalpy of melting, ΔH_c, the measured enthalpy of cold crystallization, and $\Delta H_m^{100\%}$ the enthalpy of melting ρερ μολ οφ ρεπεατινγ υνιτ οφ τηε περφεct crystal of infinite size. This latter feature has been estimated by Fischer at 93 $J.g^{-1}$ [80] for PLLA. This value is the one most often used but other values can be found in literature (146 $J.g^{-1}$ [81], 91 $J.g^{-1}$)[82].

$$X_C = \frac{\Delta Hm - \Delta Hc}{\Delta H_m^{100\%}} \tag{8.1}$$

PLA crystallizes usually between 83 and 150°C but its fastest rate of crystallization occurs between 95 and 115°C [83]. The value of the crystallization half time ($t_{1/2}$) varies according to author. In the temperature range 95–115 °C the $t_{1/2}$ of PLLA for crystallization from the melt varies between 1.5 min to 5 min [45, 79, 84]. Nevertheless the optimum, 1.5 min, is obtained at around 110°C for isothermal crystallization from melt (Figure 8.6) [45]. Not only does the $t_{1/2}$ of PLA depend widely on the crystallization temperature, but it is also linked to the crystallization type (isothermal or non-isothermal, from cold or melted state). So upon isothermal crystallization from the cold state, $t_{1/2}$is below 2 min [79, 85, 86]. Eventually, upon non-isothermal crystallization, $t_{1/2}$ also lies around 2 min [85, 87, 88]. The further the isothermal crystallization is from this optimum, the more $t_{1/2}$ increases. For isothermal crystallization below 90°C or above 130°C, $t_{1/2}$ can be beyond 10 min [45, 69].

Upon isothermal crystallization, the spherulite growth rate of PLA (96% L) is between 0.2 and 3 $\mu m.min^{-1}$ depending upon the crystallization temperature according to the authors [76, 86, 89-91], with an optimum around 115°C [76]. Moreover, when the molecular weight is divided by a factor 5, the maximal growth rate increases from 3 to 7$\mu m.min^{-1}$ at 115°C [92]. The spherulite growth rate is increased by the stereoregularity of PLA. Di Lorenzo *et al.* have shown

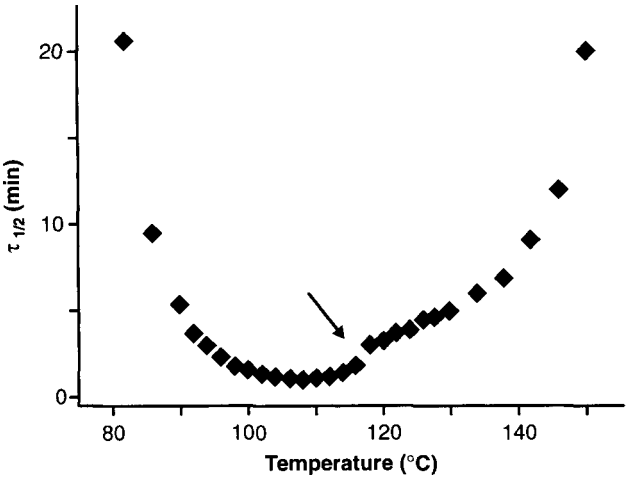


Figure 8.6 Half-time of crystallization (t1/2) of PLLA as a function of isothermal crystallization temperature reproduced with permission from [45]. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.

in Figure 8.7, two optima, one at 117°C where the rate reaches 10 μm.min^{-1} and another at 130°C where the rate is 7 μm.min^{-1} [45].

The isothermal crystallization kinetics is studied mainly with the Avrami equation (logarithmic form in equation 8.2), where $\phi(t)$ is the relative crystallinity, n_a and K_a are the Avrami exponent and crystallization rate constant respectively.

$$\ln\left[-\ln\left(1-\theta(t)\right)\right] = \ln K_a + n_a \ln t \tag{8.2}$$

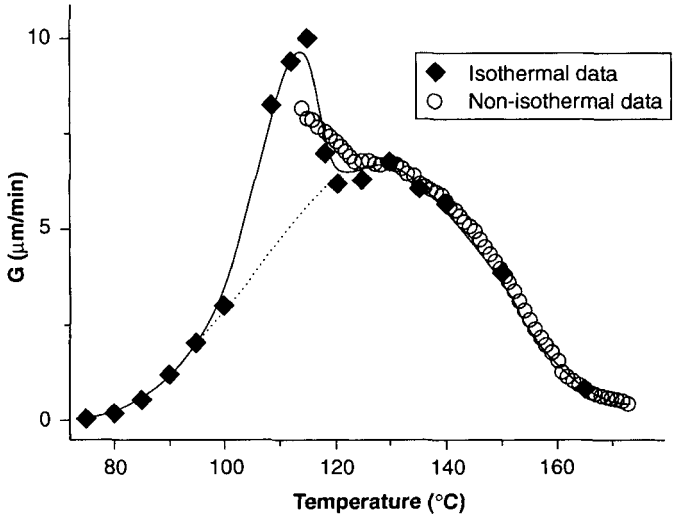


Figure 8.7 Spherulite growth rate of PLLA measured in isothermal and nonisothermal conditions reproduced with permission from [45]. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.

The kinetic studies of the isothermal crystallization of neat PLA showed that n_a is around 1.8–2.6 between 80 and 135°C [86, 93, 94]. However, Lai *et al.* observed a n_a of 3.98 at 124°C. [95]. In the same manner, the crystallization rate constant varies widely between 5.84×10^{-5} and 0.9 min^{-1} [85, 86, 94].

Upon non-isothermal crystallization the Avrami exponent takes on values between 2.1 and 4.82, whereas the Avrami crystallization rate constant are found between 0.0104 and 0.685 [85, 87].

Whatever the crystallization type, the kinetic study of the PLA crystallization, according Avrami, showed a two-dimensional or three-dimensional growth process, depending on the temperature, from a predetermined nucleation with a combination of sporadic nucleation [96, 97].

8.4.4 Melting of PLA

The melting temperature (T_m) of PLA occurs between 130 and 180°C according to the L-lactide content and the crystals formed during crystallization. The presence of meso-lactide in the PLA structure induces a decrease in the melting temperature according to the equation 8.3 where W_m is the meso-lactide fraction in the matrix and 175 is the melting temperature of PLLA [5].

$$T_m(°C) = 175 - 300W_m \tag{8.3}$$

It is therefore possible to widely reduce the melting temperature by adding D-lactide in the polymeric structure. Nevertheless the melting temperature is also dependent on the molecular weight according to Tsuji and Ikada, and T_m increases with increasing M_w to an asymptotic value [78]. The melting temperature of PLLA can be increased 40–50°C up to 200°C by physically blending the polymer with PDLA. [38].

The melting peak is simple or double according to the crystalline forms and the lamellae thickness of the spherulites using the Gibbs-Thomson relation as shown in equation 8.4.

$$T_f = T_f^{\infty}\left[1 - \frac{2\sigma_e}{L_c \Delta H_f^{\infty} \rho_c}\right] \tag{8.4}$$

The melting temperature for the thermodynamic equilibrium, T_f^{∞}, representing the melting point of an infinite size and molecular mass crystal, equals 207°C for PLLA [10]. The crystal density, ρ_c, is reported to be 1.29 kg/L and the surface energy for the extremity of the lamellae, σ_e, is 53.6×10^{-11} $J.m^{-2}$.

8.5 Mechanical Properties of PLA

The mechanical properties of PLA, which have been extensively studied, are dependent on the production process and the amorphous or semi-crystalline state of the sample. Amorphous PLA presents a tensile modulus between 2.05

and 3.25 GPa [47, 98-103], except according to Murariu *et al.* who showed a PLA with a 1.02 GPa tensile modulus [104]. This considerable range is due to the tensile test rate which varied from 1 $mm.min^{-1}$ to 50 $mm.min^{-1}$ for the different studies. The yield stress and the strain at break lie between 32 and 68 MPa and between 3 and 20%, respectively [47, 89, 101-104, 118]. The crystallization of neat PLA slightly modifies the mechanical properties. According to Kulinski and Piorkowska [89], the cold crystallization induces a slight decrease in the yield stress and strain at break. However Perego *et al.* [105] did not notice this modification in the stress and strain, whereas they showed an increase in the modulus of elasticity and in the impact resistance, presumably due to the crosslinking effects of the crystalline domains. Perego *et al.* [105] also showed an influence of the molar mass of the amorphous polymer on the impact resistance and the flexural strength which was multiplied by 1.5 to 2 when the molecular mass was raised from 23 000 to 66 000 $g.mol^{-1}$. These evolutions are the same when the samples are annealed.

The comparison of two grades of PDLLA, one for general purpose and the other one for injection, showed a possible difference in the modulus which can be due to a molecular mass discrepancy. Moreover, the comparison of the mechanical properties of oriented and unoriented films depicted an increase in the elongation at break with orientation.

At room temperature, the mechanical properties of PLA are close to the one of PS but smaller than the one of PET (Table 8.5). Polyolefins present reduced stress at yield compared to PLA but the strain at break of LDPE and HDPE are much higher than the one of PLA. Compared to another biobased polymer, poly(hydroxybutyrate) (PHB), PLA shows better mechanical properties with higher modulus of elasticity and stress at yield.

The influence of plasticizers on the mechanical properties of PLA has been extensively studied. Various plasticizers have been tested with PLA such as, glycerol, PLA oligomers, poly(ethylene glycol) monolaurate [98],

Table 8.5 Comparison of mechanical properties of PLA to synthetic polymers.

Polymer	Modulus of Elasticity (GPa)	Stress at Yield (MPa)	Strain at Break (%)
PET[106]	2.8-4.1	275	60-165
Bioriented PS[106]	3.2	55-82	3-40
HDPE[107]	-	38 ± 11	586 ± 47
HDPE[108]	0.95	24,5	
LDPE [107]	-	25 ± 7	706 ± 77
PP[109]		44,5	20
PLA (98% L-lactic acid)[106]	2.11	72	10,7
PHB[110]	0.911 ± 0.020	31.0 ± 0.3	7.0 ± 0.1

triacetine [104, 111], diethyl bishydroxymethyl malonate [112], poly(1,2-butanediol), dibutyl sebacate, acetyl glycerol monolaurate [99], poly(propylene glycol) [113, 114] and polyadipates [102, 104, 115]. However, only a few substances brought about substantial improvements of mechanical properties. The two most used plasticizers are poly(ethylene glycol) (PEG) [46, 89, 98-101, 116, 117] with variable molecular masses and citrate derivatives, in particular acetyl tributyl citrate (ATBC) [46, 104, 111, 117-119]. To observe significant modification of the mechanical properties, the plasticizer content in PLA should be around 20%. After plasticization, the modulus of elasticity and the yield strength decrease, whereas the stress at break increases dramatically. At 20% ATBC content in PLA, the material presents a strength at break between 23.1 and 30 MPa, a modulus of elasticity between 0.1 and 0.27 GPa and a strain at break above 298% [46, 104, 118]. As ATBC, PEG induces an increase in the stress at break and a decrease in the strain in the modulus of elasticity. However, at a concentration which is dependent on the molecular mass, a phase separation is observed. The higher the molecular weight, the lower the plasticizer content is at which a phase separation is observed. For this reason, the low molecular mass of poly(ethylene glycol) is preferentially used for plasticization. The addition of PEG at 20%, whose molecular mass is 400 $g.mol^{-1}$, leads to a strength at break of around 16 MPa, a strain at break between 21.2 and 71%, and a modulus of elasticity around 0.5-0.6 GPa [46].

8.6 Barrier Properties of PLA

The barrier properties of PLA have not been extensively studied. The first articles treating the permeability of PLA film have been published in 1997 [120, 121], when PLA started to be considered for packaging applications. PLA films with various L/D ratios, different crystallinity degree and blends with numerous additives and polymers have been tested in recent years with gases, water vapour and organic compounds.

8.6.1 Gas Barrier Properties of PLA

The oxygen permeability of amorphous PLA has been reported to be between 1.3 and 2.0 $\times 10^{-18}$ $m^3.m.m^{-2}.s^{-1}.Pa^{-1}$ at 30°C depending on the L/D ratio. Indeed, the increase in the L-lactide content, between 50 and 98.7%, causes an increase in the solubility coefficient leading to the increase in the permeability [122, 123]. However, this modification of the permeability coefficient seems to be insignificant between 94 and 98% L-lactide content at 25°C [106, 124]. Figure 8.8 shows that whatever the L/D ratio, the oxygen barrier properties of PLA are intermediate between low oxygen barrier film (LDPE, PS) and good oxygen barrier films (unplasticized PVC and PET).

The oxygen transport coefficients of PLA, whatever the L/D ratio, are sensitive to the measurement temperature and water activity. Auras *et al.* [106] showed that the increase in the temperature leads to an increase in the oxygen

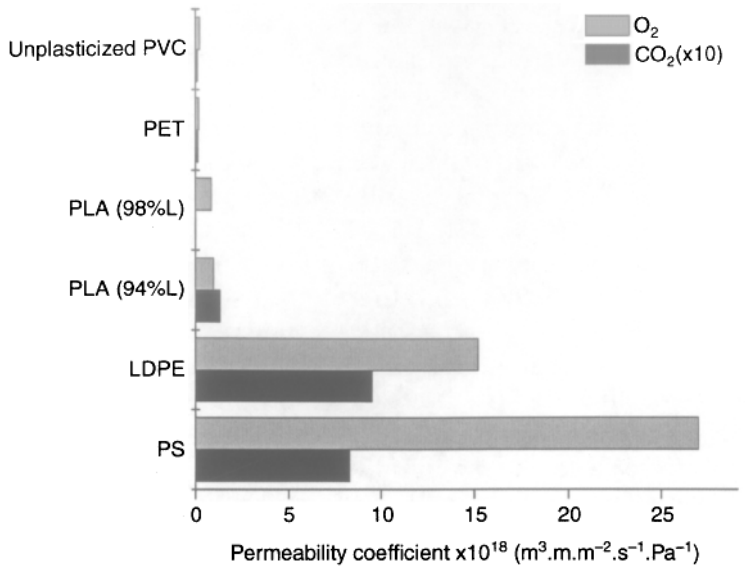


Figure 8.8 Oxygen and carbon dioxide barrier properties of PS, PET, LDPE, unplasticized PVC and PLA at 25 °C, data from [122, 125, 126].

permeability and solubility coefficients in dry conditions. However, the increase in the water activity also induces an unexpected decrease of these two coefficients [106]. On the contrary, the diffusion coefficient shows an increase with the water activity at each temperature [49]. This behaviour is attributed to the plasticization effect on the amorphous phase by water molecules.

The effect of crystallinity has also been studied with PLA [125] containing different L/D ratio [106]. The poly(98% L-lactide) with a higher crystallinity degree displays lower permeability coefficients than those of the poly(94% L-lactide). This effect has been confirmed by Drieskens *et al.* [127] who studied the effect of annealing on PLA transport coefficients. They showed that an increase in crystallinity induces a progressive decrease in the permeability coefficient. The diffusion coefficient first gradually decreased (between 0 and 10% of crystallinity) and then more sharply, while the solubility coefficient seemed to increase slowly with the crystallinity of PLA. Drieskens *et al.* [127] explained this behaviour in the first phase of the crystallization, by an increase in the tortuosity, due to the presence of spherulites. In the second phase, improvement of the internal crystal structure may have induced the further decrease in the diffusion coefficient. The increasing crystallization may be accompanied, according to the authors, with a dedensification of the amorphous phase which may lead to the unexpected increase in the solubility coefficient of PLA [127]. An overall decrease in the oxygen permeability with increasing crystallinity has also been observed by Sawada *et al.* [128]. Analysis of the experimental data showed the diffusion coefficient increased apparently for small crystallinity degrees and the solubility coefficient decreased over the whole range of tested crystallinity. Colomines *et al.* [125], showed even a slight increase in the oxygen permeability of PLLA upon moderate crystallization,

and not unlike Driskens *et al.* [127], they suggested that dedensification was one of the causes of this unusual behaviour. Sawada *et al.* [128] proposed a different hypothesis suggesting a continuous space at the interface between crystalline and amorphous domains, and this space, larger than gas molecules at low crystallinity degrees, could facilitate the gas diffusion in PLA.

The addition of plasticizer used for the improvement of mechanical properties leads generally to an increase in the oxygen permeability coefficient due to the higher mobility of the polymer chain and higher free volume [102]. On the contrary, the dispersion of nanoclays in PLA makes it possible to divide the permeability coefficient by 2 or 3 depending upon the type of the nanoclays (e.g. organomodified montmorillonite, cloisite 25A or 30B, organomodified synthetic fluorine mica) and exfoliation [129-131].

The carbon dioxide permeability coefficient has been measured at 1.1×10^{-17} $m^3.m.m^{-2}.s^{-1}.Pa^{-1}$ at 25 °C [106] and as previously observed in the case of oxygen, increases with the L content in PLA (Figure 8.8) and the temperature [106].

The crystallization of the PLA matrix induces a decrease in the carbon dioxide permeability coefficient [123, 132]. According to Sawada *et al.*, [128] the effect of crystallinity on CO_2 permeability is similar to the one on oxygen, that is to say there is a slight increase in the diffusion coefficient at low crystallinity followed by a decrease of 40%, whereas solubility decreases slowly in relation with the crystallinity degree.

The nitrogen transport coefficients of PLA are lower than those for oxygen [123]. Moreover the reported value of nitrogen permeability, around 3.8×10^{-19} $m^3.m.m^{-2}.s^{-1}.Pa^{-1}$ [122], is lower than the value for crystalline PS and LDPE (5.9 and 7.3×10^{-18} $m^3.m.m^{-2}.s^{-1}.Pa^{-1}$) [122, 133] at 25°C but higher than the one of unplasticized PVC (8.9×10^{-20} $m^3.m.m^{-2}.s^{-1}.Pa^{-1}$) [122].

The L/D ratio might not affect the nitrogen permeability of PLA [134]. The crystallinity effect has also been evaluated for this gas and it seems that the nitrogen permeability coefficient decreases with the increase in the crystallinity degree of PLA [128].

The gas selectivity in the PLA membrane depends on the chosen gas. Lehermeier *et al.* [124] show that the separation factor of CH_4 and CO_2, equal to 10, is not crystallinity and L/D ratio dependent. Only change in temperature makes it possible to increase this factor. Sawada *et al.* [128] confirm the inefficiency of the crystallinity on the permselectivity but also show that the diffusivity and the solubility selectivity are not influenced by crystallinity. The gas permselectivity in PLA is larger than 120 for H_2/N_2, 6 for O_2/N_2, 23 for CO_2/N_2, 27 for CO_2/CH_4, and 1 for CH_4/N_2 [128]. The permselectivity of PLA is two times larger than those of LDPE and PVC for O_2/N_2 and CO_2/N_2 [122].

8.6.2 Water Vapour Permeability of PLA

The water vapour permeability of amorphous PLA varies from 1.8 to 2.3×10^{-14} $kg.m.m^{-2}.s^{-1}.Pa^{-1}$ at 25°C [135-137]. As shown in Figure 8.9, the PLA data are always lower than the one of PCL but higher than the one of PET, PS and PHBV.

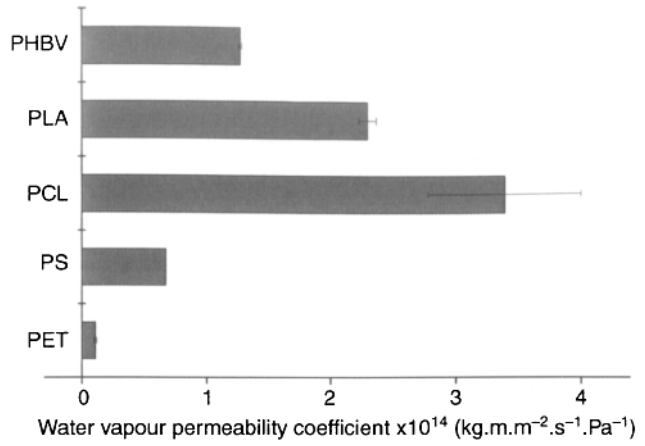


Figure 8.9 Water vapour permeability coefficient of PHB, PLA, PCL, PS and PET at 25 °C [5, 137].

Siparsky *et al.* [121] showed that the increase in the L-lactide content in PLA induced no significant modification of the water vapour permeability whatever the temperature or relative humidity, except a decrease in the permeability coefficient at 90% RH between 50 and 90% of L-lactide content. This was not confirmed by Tsuji *et al.* and Auras *et al.* who showed a constant permeability, around 1.8-1.9×10^{-14} kg.m.m^{-2}.s^{-1}.Pa^{-1} at 20°C and 40–90% RH, despite an increase in the L-lactide content [106, 136].

The crystallinity degree does not reveal any change in the solubility coefficient whatever the temperature and the relative humidity are [120]. Consequently the permeability coefficient seems to be controlled by the diffusion coefficients which increase with the crystallinity degree at certain temperature and relative humidity [121]. This is in contradiction with the decrease in permeability coefficients, reported by Tsuji *et al.* and Shogren [120, 136]. Indeed, in their study the diffusion and solubility coefficient decrease slightly with the crystallinity degree of PLLA [138].

Furthermore, the effect of the temperature on measured water vapour permeability changes in function of the study. Auras *et al.* showed a decrease of the permeability with the temperature, whereas Shogren highlighted an increase [5, 120]. Siparsky *et al.* [121] showed that the diffusion coefficients increase and the solubility coefficients decrease with the temperature at 90% RH, which is contradicted by Holm *et al.* who showed higher values of moisture sorption at a higher temperature [139]. The various and contradictory results show that the mechanism of water transport in PLA is not completely understood yet [5]. In particular the presence of water clusters in the PLA matrix and their potential to diffuse in cluster form are still controversial.

8.6.3 Permeability of Organic Vapours through PLA

The transport of organic compound in PLA has been the subject of only a few studies, but is of importance in food packaging applications. Ethylene,

a compound accelerating fresh food ripening, plays an important part in the storage of fresh fruits. The ethylene permeability of amorphous PLA has been tested and evaluated at 6.8 $\times 10^{-18}$ $m^3.m.m^{-2}.s^{-1}.Pa^{-1}$ [124]. This value is lower than the ethylene permeability of PET (3.0 $\times 10^{-20}$ $m^3.m.m^{-2}.s^{-1}.Pa^{-1}$) [124] but higher than the value of LDPE (2.2 $\times 10^{-17}$ $m^3.m.m^{-2}.s^{-1}.Pa^{-1}$) [133]. Increasing the crystallinity in PLA induced a decrease in the ethylene permeability [124, 128].

The transport coefficients of ethyl acetate, an aroma booster found in a large variety of aroma formulations, have been calculated from sorption of ethyl acetate in PLA experiments. The ethyl acetate permeability of PLA is 5.34 $\times$ 10^{-19} $kg.m.m^{-2}.s^{-1}.Pa^{-1}$ at 30°C and 0.3 activity. It is higher than the one of PET but lower than those of PP and LDPE. However, the ethyl acetate solubility coefficient in PLA, equal to 6.17 $\times 10^{-3}$ $kg.m^{-3}.Pa^{-1}$ at 30°C and 0.3 activity, is higher than the other polymers [140]. This result is comparable to the value reported by Colomines *et al.* for an amorphous PLA with 99% L-lactide content at 25°C and 0.5 activity [125]. Moreover, increasing the crystallinity of PDLLA provokes a decrease of the ethyl acetate solubility coefficient at 0.5 and 0.9 activity [125].

The permeability of limonene through PLA, has been estimated by Auras *et al.* [140]. at a maximal value of 9.96 $\times 10^{-21}$ $kg.m.m^{-2}.s^{-1}.Pa^{-1}$ at 45°C and with a limonene partial pressure of 258 Pa. The permeability value of this more hydrophobic molecule is lower than that of ethyl acetate in PLA and is lower than those measured for PET, PP and LDPE [140]. Haugaard *et al.* have confirmed the low limonene sorption in PLA by comparison with the one in HDPE [141].

The study of scalping aroma compounds by PLA during high pressure treatment highlighted the lower PLA uptake of organic molecules compared to the one in LDPE. Indeed, ethyl hexanoate and limonene are more sorbed in the more apolar matrix, LDPE, than in PLA. On the contrary, the more polar molecules, 2-hexanone and ethyl butanoate, are more sorbed by the more polar polymer matrix, PLA [142].

Consequently, it appears that the L/D ratio of PLA influences the gas permeability but no conclusion can be reached regarding the water vapour transport in this polymer. Moreover, generally speaking, the crystallization of the PLA matrix makes it possible to decrease the gas permeability and the organic compound sorption in PLA. However, no agreement has been formed regarding the influence of crystallinity degree on water vapour transport in PLA due to the variety of PLA composition (L-lactic acid content) and measurement systems.

8.7 Degradation Behaviour of PLA

The degradation behaviour of PLA has been subject to extensive study, because it is one of the primary functions of the polymer giving rise to applications. Hence, degradation can be either desired, in the cases of biomedical applications or for biodegradation, or unwanted when it takes place during processing. Degradation of PLA can be either abiotic or biotic, the latter being a process involving biocatalysts.

8.7.1 Thermal Degradation

Thermal degradation is an abiotic process taking place mainly during processing, and is therefore highly undesirable. Generally speaking, aliphatic polyesters as PLA have no high thermo-stability. Degradation processes can already start at temperatures as low as 215°C [143], while main degradation is observed by thermogravimetric analysis to extend between 215 and 370°C [143-145]. The thermal decomposition peak lies at approximately 360°C [143-145]. Carrasco *et al.* [145] observed moreover, that thermal stability increased with increasing molecular weight. In the case of the α-hydroxyester PLA, it was concluded that the carbonyl-carbon – oxygen linkage was the most likely to break upon heating [146] and the reaction kinetics being of first order [144]. The mechanisms of thermal degradation are rather complex, involving thermohydrolysis by trace amounts of water, zipper-like depolymerisation, inter- and intra-molecular transesterification, and oxidative random main chain scission [19]. Moreover, reactive end groups, residual catalyst [143, 147], unreacted starting monomer and impurities have been reported to enhance the PLA thermal degradation [143, 148]. Hydrolysis of PLA during processing is one of the main factors of decrease in molecular weight, as observed by several authors [149-151]. Thus, careful drying of PLA granules is of importance in the extrusion process for minimizing process temperature and residence time. Kopinke *et al.* [143] proposed that PLA also degrades through intra- and interchain transesterification, in accordance with the results of McNeill and Leiper [152], through cis-elimination and through radical and non-radical reactions, which yields CO, CO_2, acetaldehyde, methylketene, acrylic acid, and acyclic oligomers. McNeill and Leiper [152] and Jamshidi *et al.* [81] showed the importance of non-radical backbiting ester exchange involving terminal hydroxyl groups. Additives, such as deactivators of remaining catalysts or the derivation of hydroxyl end groups can increase PLA melt stability [81, 152, 153]. Tsuji and Fukui [154] showed that the formation of a stereocomplex PDLA/PLLA enhances the thermal stability below 260°C. Beyond that temperature, no advantage for the blended films was found, which the authors attributed to the breaking of the stereocomplex, and thus to returning to the properties of single PLA chains.

8.7.2 Hydrolysis

Hydrolysis is the main route to PLA degradation. It can be either (i) abiotic and undesired, as in the case of thermohydrolysis during processes, or (ii) abiotic and desired, as in the case of hydrolysis under physiological conditions in biomedical applications, or (iii) biotic and desired, as in the case of biodegradation by microorganisms. Hydrolysis kinetics of PLA in mild temperature conditions ($T < 40$ °C), as they exist in a physiological environment, are of dramatic importance for the design of products, such as surgical implants or drug delivery devices. Upon immersion in an aqueous environment, water penetrates only into the amorphous phase, and cannot penetrate the crystallites. This yields primary degradation of amorphous domains, therefore

hydrolysis is considered to be a bulk erosion process [155]. In the presence of water and a catalyst, ester hydrolysis occurs, bringing about a decrease of molecular weight. Once oligomers are small enough to dissolve, mass loss of the polymer sample is observed [156, 157]. Ester hydrolysis can be either acid- or base-catalyzed. De Jong *et al.* [158] recently studied the degradation of PLA oligomers as a function of pH. They proposed the following mechanism shown in Figure 8.10.

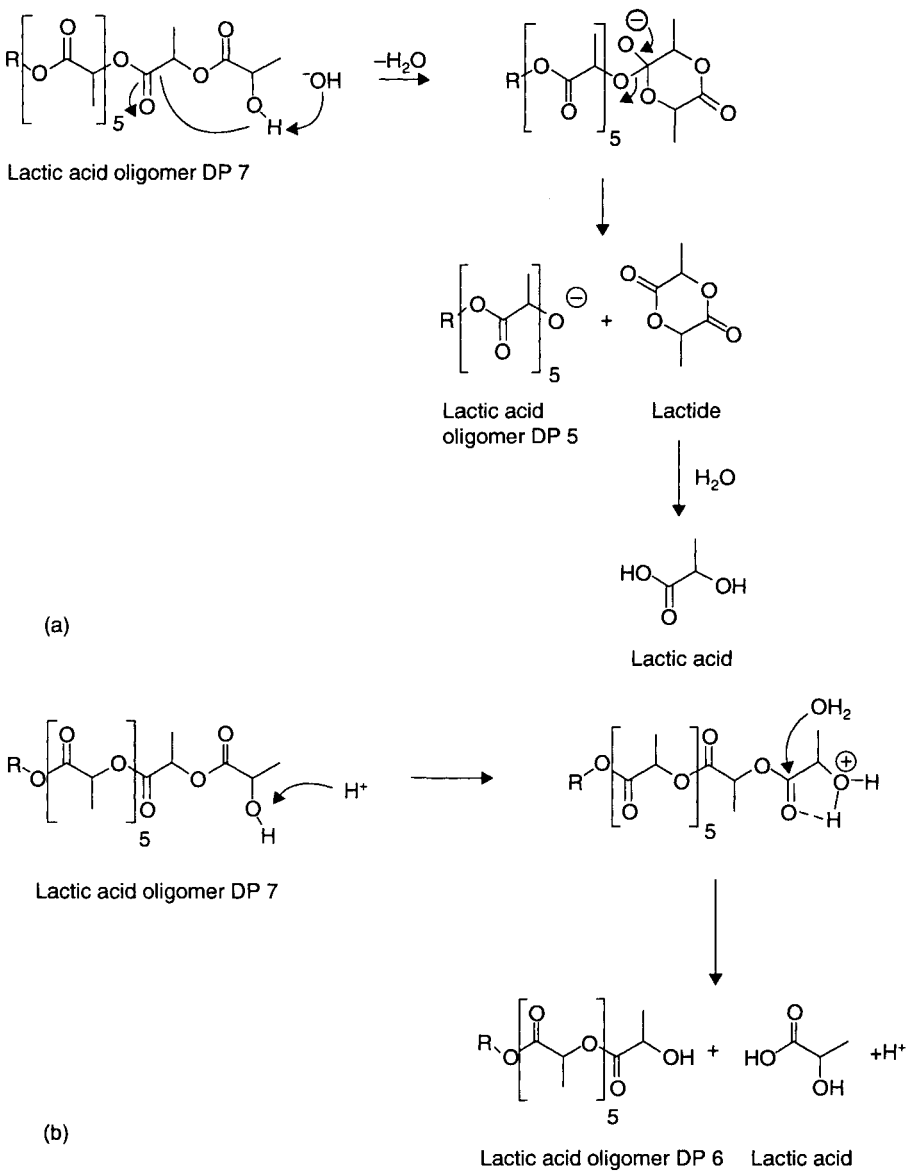


Figure 8.10 Suggestion for hydrolysis mechanisms of PLA in alkaline solution (a) and in acid solution (b) reproduced with permission from [158].

Before the polymer sample loses weight, carboxylic acid end groups accumulate in the amorphous phase, having an autocatalytic effect. Therefore, the degradation of amorphous samples proceeds more rapidly in the centre than at the surface of the specimens [156]. Degradation kinetics are furthermore dependent on initial crystallinity and, of course, temperature. Moreover, degradation induces crystallization of PLA [156]. For example, the complete degradation of PLLA Bioscrew can take 4 years [159]. PLLA rod specimens were tested *in vivo* and *in vitro* under physiological conditions. No weight loss was obtained *in vivo* for 44 weeks [160], while the polymer was degraded in 25 days at 70°C and a similar pH *in vitro* [161]. However, the use of high temperatures in order to predict the degradation kinetics needs to be carefully validated, because activation energies change significantly beyond the glass transition of the polymer [162].

There has been evidence of enzymatic degradation, where the main mechanism is hydrolysis, in microbial proteinases, esterases and lipases. Different enzymes are described in a recent review [40]. MacDonald *et al.* [163] investigated the effects of stereochemistry and crystallinity on the degradation of PLA by proteinase K. The enzyme showed a large substrate tolerance. Amorphous films of PDLLA with L-lactide contents ranging from 80% to 95% exhibited film weight loss rates that were nearly identical. Proteinase K was, however, sensible to the unit distribution of the D and L stereoisomers. Films prepared by L-lactide/meso-lactide copolymerization showed slower weight loss rates compared to L-/D-lactide copolymerizations. Moreover, proteinase K proved to be very sensitive to crystallization, which strongly decreased the degradation kinetics. Biaxial orientation also has a retarding effect on the enzymatic degradation of PLLA by proteinase K, which also seems to be more important than the effect of crystallinity [164]. Different hypotheses were formulated to explain this observation: A diminished attachment of the enzyme to strained chains, decreased cleavage of strained chains, or the observation that action was located exclusively at the surface, while in non-oriented samples the degradation proceeded beneath the spherulitic crystalline residues [164].

8.7.3 Biodegradation

Biodegradability of polymers is a multifunctional property referring to end-of-life options of materials in different biological environments (e.g. composting, anaerobic digestion). According to the ATSM standard D-5488-94-d, biodegradation is defined as being "a process capable of the decomposition of materials into carbon dioxide, methane, water, inorganic compounds, or biomass in which the predominant mechanism is the enzymatic action of microorganisms, that can be measured by standard tests, in a specified period of time, reflecting available disposal conditions." Biodegradation can therefore involve different mechanisms, such as dissolution, hydrolysis and enzyme-catalyzed degradation, but also oxidation, photolysis or radiolysis. In the case of PLA, an abiotic (catalyzed) hydrolysis step leads to the decrease of the molecular weight of the polymer. Once oligomers form, they are able to dissolve and be attacked and assimilated by microorganisms, which will metabolize them into

CO_2 and water under aerobic conditions [5, 157]. Biodegradation of PLA has been studied in soil, in sea water and in compost. It has been found, not surprisingly, to depend on the structure (molecular weight, stereochemistry) and the crystallinity of the polymer. Increase in molecular weight and crystallinity considerably slow down biodegradation [165]. Furthermore, environmental conditions, such as temperature and water availability have a strong influence on biodegradation time. Biodegradation of PLA has been studied in different environments. In soil activation the time can be very long. For example, in one study no degradation was observed within 6 weeks [166], in another study a weight loss between 20 and 75% in function of PLA stereochemistry was found after 20 weeks [167]. Tsuji *et al.* [168] investigated the degradation of aliphatic polyesters in sea water. In static laboratory conditions no weight loss of PLLA could be observed in 10 weeks, elsewhere the weight loss was accelerated upon immersion directly into the sea inside mesh grids. However, loss of particles due to mechanical shear and breakdown could not be excluded in this case [169]. Many studies of PLA biodegradation in the composting environment were carried out. Ghorpade *et al.* [170] showed full degradation of PLA in compost mixed with yard waste within 30 days at 52°C, although high PLA concentration (30% w/w) retarded the biodegradation because of the drop in pH in the compost. Figure 8.11 shows the biodegradation of PLA bottles in real compost conditions [171]. PLA bottles were shown to biodegrade to 80% upon 58 days at 58°C under simulated composting conditions according to ASTM and ISO standards [172]. Furthermore, creating composite structures with PLA has been shown to accelerate biodegradation. Some examples are the increase of the biodegradation kinetics for PLA/wheat straw and PLA/soy straw composites [173] or PLA/layered silicate nanocomposites [174].

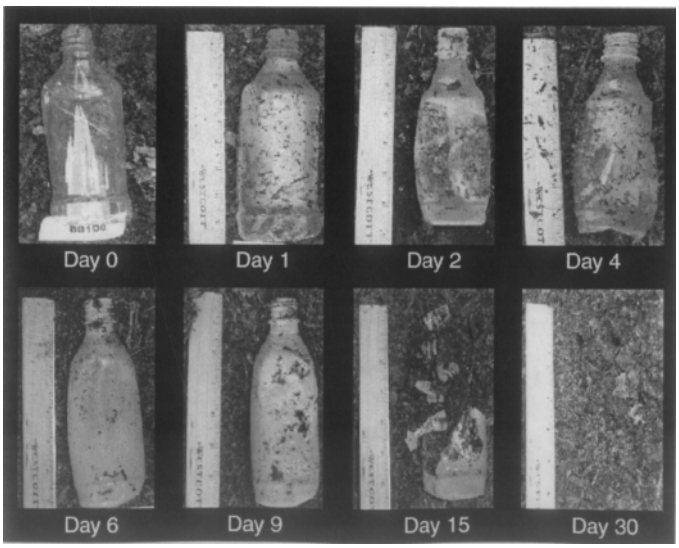


Figure 8.11 Biodegradation of PLA bottles in real composting conditions reproduced with permission from [171]. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.

However, although the initial chemical hydrolysis process has been extensively studied and well accepted, a clear understanding of the microbial degradation processes is still missing [175]. It has been suggested that PLA degradation may even be an entirely abiotic process [176]. Suyama *et al.* [177] investigated microbial populations from soil capable of degrading aliphatic polyesters, such as PHB or poly(caprolactone). However, no PLA degrading strain was found. This suggests that PLA degrading microorganisms are not widely distributed in natural environments. In a mini-review Tokiwa and Calabia [165] published a list of several PLA degrading strains, highlighting the importance of actinomyces or thermophilic strains of the genera of *Bacillus*. Sangwan *et al.* [175] recently proposed a study indentifying PLA-degrading microorganism from PLA enriched compost with the help of molecular ecological techniques, showing the importance of fungi of the genera *Thermophylospora*, *Thermomonospora*, and *Paecilomyces*. This study also suggests that several numbers of PLA degrading strains might not be readily cultivable with laboratory techniques and are therefore difficult to identify. Further work certainly will be required using both screening isolation strategies as well as molecular ecological techniques for direct identification in order to gain understanding of PLA degradation and exploit the microbial potential for biotechnological applications.

8.8 Processing

PLA processing has recently been the subject of an excellent review by Lim *et al.* [178]. PLA has been processed by a large variety of transformation methods: extrusion, extrusion film sheeting, extrusion casting, extrusion blowing, injection moulding, stretch blow moulding, thermoforming, foaming, and fibre spinning. A general point to look out for in the thermal processing of PLA is its susceptibility to thermal hydrolysis. Therefore, PLA pellets need to be carefully dried, typically to less than 100 ppm [178]. Suppliers recommend drying to at least 250 ppm moisture content before extrusion. Commercial PLA resins can be processed on conventional extruders equipped with a general purpose screw of L/D ratio of 24–30, or on screws for PET processing. The recommended compression ratio is 2–3, and the heater setpoint usually between 200-210°C [178]. PLA has low melt strength, therefore horizontal roll stack configurations are preferred for film extrusion, and relatively high roller temperatures are required to prevent lactide condensation (25–50°C). Temperatures that are too high should be avoided, however, to prevent sticking to the rollers. For the extrusion blow process, viscosity enhancers, mostly coupling agents are used. Additives are generally proprietary and not disclosed in open literature. One commercial example is a coupling agent of styrene, copolymerized with methyl methacrylate and glycidyl methacrylate [179].

The mechanical properties of PLA can be enhanced by orientation, generally in machine direction orientation with a draw ratio from 2 to 3, and in transverse direction with a draw ratio from 2 to 4 [178]. Especially toughness

can be dramatically improved. For example, elongation at break in machine direction can be increased to 160% upon biaxial orientation [180]. PLLA shows strain-induced crystallization upon drawing above glass transition temperature [180, 181]. Furthermore, stress-strain curves show strain-hardening behaviour, which is sensitive to the draw temperature. Structure investigation of uniaxially drawn samples showed the occurrence of a mesomorphic phase at low drawing temperature (70°C) starting at 130% strain. At higher draw temperatures (90°C) a well-defined crystalline phase developed at strains higher than 250%. In the mid-temperature range (80°C), both phases coexisted [182].

Fibre spinning of PLA has been done by melt spinning, solvent spinning, and more recently electrospinning. Lim *et al.* give a good overview of the different spinning conditions and fibre properties [178]. In general, solution spun fibres have superior mechanical properties compared to melt spun fibres, which is attributed to the lower chain entanglement in the solution state compared to the melt state.

A strategy to improve PLA properties and/or to reduce material costs is blending with other polymers or compounding for the fabrication of composites. A number of articles have been published treating PLA blends with polymers such as starch [98, 183], poly(hydroxyalkanoates) [184, 185], or poly(butylene succinate) [186, 187]. For example, PLA/thermoplastic starch blends suffer from low adhesion between the phases, causing modest mechanical properties [98]. PLA/poly(hydroxybutyrate) blends are immiscible within a wide range of conditions, and an improvement of mechanical properties can be observed [188]. Small quantities of Nodax™, being a poly(hydroxybutyrate) copolymer [184] or of chemically modified poly(hydroxyalkanoates) [185] improved toughness of PLA. PBS was found to have a nucleating effect on PLA upon blending, yielding higher crystallinity and enhanced cold crystallization [186, 187].

Various composite materials of PLA have been prepared in order to overcome mechanical limitations and to decrease material costs. Among different filler materials, minerals and materials derived from renewable resources have received the most interest. The reinforcing effect of a filler depends mainly on interfacial adhesion between filler and matrix and on its dispersion in the polymer matrix. In most cases the effective dispersion requires twin screw extruder mixing. For example, PLLA-cellulose composites were produced with microcrystalline cellulose, cellulose fibres and wood flour by twin screw extrusion and injection moulding. Wood flour and microcrystalline cellulose had a better nucleating effect on PLLA than cellulose fibres, resulting in very high crystallinity degrees upon slow cooling and reheating (66%) [189]. Microcrystalline cellulose composites also maintained transparency of the PLA film and enhanced barrier properties [190]. An increasing number of articles has been published in recent years on nanocomposites of PLA, and on nano-biocomposites which have been recently reviewed by Bordes *et al.* [191]. For example, PLA-nanocomposites have been extensively studied by Shina Ray and Okamoto [192-194]. They successfully prepared PLA-nanocomposites

using melt-extrusion processes. Nanofillers enhanced PLA properties, such as mechanical moduli, thermal stability, crystallization, gas barrier properties, and biodegradability.

8.9 Applications

As claimed on the website of Omnexus in 2010 [195], many corporations have adopted as part of their corporate objectives the need to develop sustainable raw materials. The use of legislative instruments is a significant driving influence for the adoption of bioplastics. In Europe and Japan, the automotive and packaging sectors are the most affected by legislation. The Packaging and Packaging Waste Directive 94/62/EC and the End of Life Vehicle Directive 2000/53/EC are two examples of such legislative drivers. Increasing oil prices, depleting oil reserves, biodegradability/total life cycle impact from sustainable resources and the use of legislative instruments are major driving forces for the use of biopolymers by corporations. Moreover, the suitability of material properties for converters, the technical feasibility of processing options, the versatility of applications and ultimately, commercial viability of production and processing, are the key factors which will decide actual use of biodegradable polymers [195].

In this context, PLA is the most mature and versatile polymer derived from a natural source that can be processed by the existing technologies used for petroleum-based polymers. By tuning the molecular weight and its copolymerisation with other polymers, by controlling several factors affecting the lifetimes and degradation rates, including molecular mass, crystallinity and additives, the tailoring of PLA properties can be achieved leading to a large range of applications.

In 2010, the European Polysaccharide Network of Excellence (EPNOE) prepared a research road map vision to 2020 focusing on polysaccharides used in material structuring [196]. This report was completed with a market study on biomass-based polymers and products reviewed by Shen *et al.* [41]. Future and present applications have been reported by the two leading producers of PLA (NatureWorks LLC and Purac) at Horizon 2020. It clearly appears that the most promising sectors are the textile, automotive and building sectors, which require durable PLA products (Figure 8.12).

In the past and nowadays, biomedical and food packaging applications sectors have been sources of numerous developments:

8.9.1 Biomedical Applications of PLA

One of the main routes to degradation of PLA is cleavage of the ester linkages by hydrolysis leading to a successive reduction in molecular weight. This degradability coupled with its biocompatibility are the reasons why PLLA has been extensively used in the past four decades for medical applications purposes including suture and scaffold for tissue engineering. High molecular

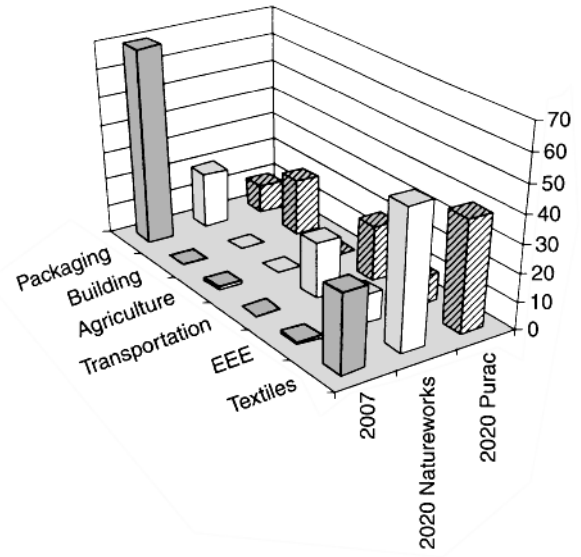


Figure 8.12 Percentage of actual and future applications of PLA, data from [41].

weight PLLA was used for orthopedic products such as bone fixation material [197, 198]. Low molecular weight with narrow molecular weight was especially desirable for rapidly degrading biomaterials such as those used for short-duration parenteral drug delivery systems [199].

Drug delivery microspheres, in particular smart systems with controlled release, have been developed with PLA and its copolymers with glycolide to produce poly[(D,L-lactic acid)-co-(glycolic acid)]. Since the 80's up until the present, many publications have reported on the various applications in the pharmaceutical field for using PLA and copolymers and polymers for their sustained release parenteral formulations and performance in drug release in a controlled manner from microspheres [200, 201].

8.9.2 Packaging Applications Commodity of PLA

While the rapid hydrolytic degradation of the PLA is an advantage for medical uses, this property becomes a main drawback for food packaging applications [202]. PLA meets many requirements as a packaging thermoplastic and has been suggested as a commodity resin for general packaging applications [203]. Since the 1990's, looking to extend PLA applications, properties such as impact strength or flexibility, stiffness, barrier properties, and thermal stability, have been studied. The extensive research to improve PLA for food packaging purposes was well reviewed by different authors [5, 15, 204]. Particular attention focused on the barrier properties concerning water vapour and oxygen by Mensitieri *et al.* [205], who showed that PLA like other biopolymers has lower oxygen barrier performances than petrochemical polymers coupled to its moisture susceptibility. To improve the barrier properties of biopolymers, several approaches are available [203]: i) Use of coating with materials which would

add hydrophobicity to the packaging material, ii) lamination of two or more biopolymers (co-extrusion), and iii) development of blends of biopolymers with different properties. Some examples include PLA/polyethylene glycol blends, PLA/polyhydroalkanoates blends and PLA/polycaprolactone blends. Supplementary possibilities are: v) Chemical and/or physical modification of biopolymers, vi) development of micro and nanocomposites based on biopolymers [191, 206].

Another major limitation of PLA relative to PET widely used in food packaging applications is due to its poor barrier properties mainly against oxygen and CO_2. For the food industry, the prediction and control of the ageing of packed food are a major goal in order to meet consumer expectations. Packaging contributes to the end quality of the product by employing appropriate barrier properties. However, the interaction of packed food and oxygen is very complex and depends on residual oxygen in the freshly packed food, along with the oxygen tolerance of the food. Table 8.6 presents the oxygen tolerance of foods, meaning how much oxygen a substrate can take up until first sensory deviations can be detected. If this oxygen tolerance is known, packaging can be developed or chosen to reach optimal storage conditions to increase shelf-life.

The packaging industry has been constantly looking to replace glass with polymeric materials and has recently focused on biobased polymers. However, for very delicate food products such as beer or coffee, there is a challenge to keep the freshness of the food that is related to the lowest increase of oxygen into the pack. Salame *et al* [207] presented the Table 8.6 and proposed a relationship that will allow a rough estimation of the shelf life t:

$$t = (p_c.L)/(P.10^{10}a.500) = p_c/R. \tag{8.5}$$

Where p_c is the O_2 sensitivity of the food given in Table 8.6, with L is the container thickness, a is the area to volume relationship of the package, R is the O_2 transmission rate of the package in air as ppm.day^{-1} and P is the O_2 permeation rate is given by equation 8.5.

Furthermore, Table 8.6 shows that some food products do not require high oxygen barrier properties. Haugaard *et al.* [209] showed that cups based on PLA were found to be as effective as high-density polyethylene cups in protecting an orange juice simulant and a dressing from quality changes during storage. Moreover, the properties of PLA may even be better with respect to the packaging of yoghurt due to the lower oxygen permeabilities of PLA cups compared to PS cups. The authors claimed that the use of PLA for packaging has been demonstrated by Danone in Germany, and Valio Ltd, a Finnish dairy company. These studies show that PLA has the potential to replace broadly used polymers in food applications, polyolefins and PS, in particular for small containers which are not recycled today.

One other drawback of PLA in the packaging sector is the heat deflection temperature at 50-60°C that will provoke poor resistance to heat and lumping of pellets during transport, storage, and processing, as well as cause

Table 8.6 Oxygen tolerance of foods, data from [207, 208].

Oxygen tolerance of food	
Food or Beverages at 25 °C	**Maximum Oxygen Tolerance Gain in ppm/year**
Beer	1-2
Canned soups	1-3
Baby foods	1-3
Finewine	2-5
Fresh ground coffee	2-5
Lyophilised food	5-15
Tomato products	8-20
Fruit juices	10-40
Carbonated soft drinks	10-40
Oils and Shortening	50-200
Salad Dressings, Peanut butter	50-200

deformation of trays, preforms, bottles, and cups during food conditioning. The melting temperature of PLLA can be increased by 40 to 50°C up to 200°C and its heat deflection temperature can be increased from approximately 60°C to up to 190°C by forming a PDLA/PLLA stereocomplex. The temperature stability is maximised when a 50:50 blend is used. At lower concentrations of PDLA (3–10%), PDLA acts as a nucleating agent and increases the crystallization rate. New PLA stereocomplex grades with enhanced heat-stability will widen the applicability in higher value applications, where hot-filling of foodstuff is required (bottles, cups) or for microwable trays [40].

Active packaging has been one of the major innovations in food packaging in the past years. Its aim is to respond to consumer expectations for freshness and high quality foodstuffs. Contrary to passive, inert packaging, active packaging promotes interactions with the food in order to extend shelf life, to improve sensorial properties, or to inhibit spoilage by microorganisms [210]. Antioxidant active packaging is a promising technology for the preservation of fat-containing foods. Lipid oxidation in dairy products, is a major cause of deterioration during processing and storage with the appearance of sensorial defects. Active packaging with natural antioxidants such as α-tocopherol has been developed in the past based on petrochemical polymers and more recently with PLA and its copolymers. Two modes of action were tested either by limiting the penetration of oxygen into the package [211, 212] or by a controlled release into the food in contact. Poly(lactide-co-glycolide) films loaded with

natural and synthetic antioxidants, α-tocopherol, or butylated hydroxytoluene (BHT) were tested in contact with milk powders and food simulating liquids. The antioxidant release was driven by hydrolytic degradation of PLGA in the first case, or by volatilisation with solid contact. This work showed valuable results on dry milk products [213].

Antimicrobial PLA films were also developed by loading nisin onto the PLA film surface [214], melt incorporation of lysozyme, thymol, lemon into the PLA films [215], or solvent casting with propolis being a natural active agent having antibacterial, antifungal and antioxidant activities [216]. However, the use of melt extrusion based processing is difficult because of the temperature sensitivity of most antimicrobial compounds, wherefore coating techniques are often employed [205].

The correct balance has to be found between the durability of the packaging needed for the preservation of packed food during its shelf-life, and the expected biodegradability at the end of the life cycle. Addition of nanocomposites in PLA can improve barrier properties for food applications and increase degradation in compost conditions [217]. On the other hand, plasticizers are commonly added to promote flexibility of PLA but degradation increases, and food shelf life is often negatively affected by increasing plasticizer content [89].

8.9.3 Textile Applications of PLA

The ease of melt processing, unique property spectrum, renewable source origin, and the possibility of composting and recycling are sources of the growing interest for PLA fibres and acceptance in a range of commercial textile sectors. In the form of fibres and non-woven textiles, PLA also has many potential uses, for example as upholstery, disposable garments, awnings, feminine hygiene products, and nappies. Textile, fibres and fabrics represent a sector that is forecast to become the first sector of use for NatureWorks LLC and Purac's productions [41]. In recent years, the polymer has been available on the market and its applicability has already been proven in a number of processes and final applications. However, to date its breakthrough is slower than expected in textile applications. The technological challenges to be solved were reviewed by Avinc and Khoddami [218, 219]. PLA fibres can be dyed with disperse dyes, like PET fibres. However, a variety of wet processing applications (pretreatment, dyeing, clearing, and subsequent finishing treatments) impart chemical and physical stresses on the PLA fibres. The development of the PLA stereocomplexes with higher thermal stability has the potential to limit the shrinkage of PLA fibres and fabrics during dying or ironing.

Special attention has been focused on the flammability and fire stability of PLA needed during ironing. Solarski *et al.* [220] showed that the incorporation of 4 wt % organomodified layered silicate (Bentone 104) improves the shrinkage properties and reaction to fire of PLA filaments. They concluded that this "nano effect" can be considered as permanent (*i.e.* the separated nanoplatelets are imbedded within the matrix), in contrast to some classical textile finishes, which are sensitive to washing.

PLA fabrics exhibit the comfort and touch of natural fibres such as cotton, silk and wool while having the performance, cost, and easy care characteristics of synthetics. PLA fibres demonstrate excellent resiliency, outstanding crimp retention and improved wicking compared with natural fibres. Fabrics produced from PLA are being utilized for their silky feel, drape, durability and water vapour permeability used to create breathability suitable for sport clothing applications [195].

8.9.4 Automotive Applications of PLA

Since the 1950's , the automotive corporations have shown a great interest in synthetic polymers (PS, PA, Polyurethanes, and PP) and nowadays plastic materials represent 50–55% and 25–30% w/w of the total mass of a car comprising the passenger compartment and the body of the car, respectively. In the last decade, composites of synthetic polymers with glass or carbon fibres emerged from the aeronautic sector, and penetrated the automotive sector with the aim of reducing vehicle weight. However, these composites with non-organic fillers are not easy to recycle. Driven by environmental concerns and triggered by EU legislation on the End of Life Vehicle (Directive 2000/53/EC), biocomposites have been developed that offer certain environmental advantages at the end of the use cycle when composites are landfilled or incinerated. European Union legislation implemented in 2006 states that 85% of a vehicle must be reused or recycled by 2015. Japan requires 95% of a vehicle to be recovered (which includes incineration of some components) by 2015 [221].

Car and part manufacturers turned their attention to natural fibres to reinforce plastic polymers. The most used natural fibres are hemp, sisal, flax, wood, and kenaf used in a biodegradable or non-biodegradable matrix. However, combining the biofibres with a biodegradable and renewable resource-based polymer such as PLA enables the creation of a completely biobased solution. One impediment in creating good bio-composites is a lack of interfacial adhesion between the natural fibre fillers and polymer matrix. Many approaches geared towards enhancing interfacial adhesion have been pursued, including the use of grafting agents and chemical modifications [221, 222].

Recently Purac [223] has developed a PLA compound with heat stability and impact strength comparable to poly(acetonitrile butadiene styrene) (ABS) in injection moulding applications based on the stereocomplex technology.

8.9.5 Building Applications

As of now, biopolymers are very rare in the building sector, although this sector might prove promising for PLA development in the future. As shown by their use in textile applications, carpet tiles and moquettes can be made with PLA fibre and would be useful in non-perennial uses such as in salons and expositions. Expandable foams are traditionally produced from fossil-based polymers (i.e. polystyrene or polypropylene) and largely used for insulation in building. In 2010, the Dutch company Synbra in collaboration with

Purac and Sulzer started the production expandable PLA named BioFoam®, a biodegradable and biobased alternative to EPS-foam in a variety of application areas, in particular insulation for building. PLA has also been used in France by Buitex to serve as the binder in Isonat Nat'isol, a hemp fibre used in building insulation. Bourbigot and Fontaine [224] reviewed the flame retardancy of PLA focusing on its use as a substitution for traditional polymers in the transportation, electric, and electronic equipment sectors, where fire hazard is an issue and flame retardancy is required. The solution and approach used for flame retarding PLA such as blending, use of conventional flame retardants or nanoparticles, and ingredients of intumescence were discussed. The authors concluded that the mechanism of action remains similar to those observed in other polymers when incorporating the same kind of additives. However, more scientific research specifically exploring the flame retardancy of PLA and its mechanisms is needed. In particular, combined with nanofillers of graphite and silica, PLA could present good mechanical and thermal properties which would improve the flame retardant properties of PLA [217].

8.9.6 Other Applications of PLA

Polylactic acid (PLA) has been proposed for use in the production of horticultural materials in order to reduce the environmental problem caused by the large quantity of plastic used in this sector, and for use as a matrix for the controlled release of herbicides. Chang *et al.* [225] evaluated the impact of PLA in the growth stimulation and yield improvement of soybeans. Greenhouse studies confirmed that both lactide and PLA increased soybean leaf area, pod number, bean number, and bean and plant dry weight. Low molecular weight polylactic acid was used as a matrix to formulate biodegradable matrix granules and films with bromacil using a melt process [226]. Low molecular weight PLA-based formulations were shown to be useful for the application of pesticides to sensitive systems such as seeds. PLA as an encapsulation matrix for herbicides or pesticides could help to reduce their environmental impact.

Within the field of durable applications, Japanese companies are using PLA and other biopolymers in cell phone and computer housings. Sanyo Mavic Media Co. Ltd. (Japan), a subsidiary of Sanyo Electric Co., Ltd., has introduced the world's first biodegradable compact disc-based PLA, including its film packaging and case. Developed jointly by Mitsui Chemicals, Inc. (Japan) and Sanyo Mavic Media, the new discs, marketed under the name MildDisc, are virtually indistinguishable from conventional discs made of polycarbonate, with no trade-off in sound or picture quality. With estimates of worldwide disc demand at more than 10 billion pieces, market opportunity is substantial. Sanyo Electric is targeting volume customers producing pre-recorded CDs, such as music CDs, video CDs, or software CD-Roms. The company is also working on recordable and rewritable versions of the MildDisc and on DVDs [195].

8.10 Conclusion

In order to achieve success in overcoming major technical challenges, the mechanical, thermal and barrier properties of PLA have been, and will continue to be improved upon, all while maintaining biodegradability. Already the versatility of PLA has been shown in various applications, proving its competitiveness with petroleum-based materials and its particular place within the family of bio-based polymers. Today more durable solutions are underway based on the success of the controlled synthesis of PLA from lactic acid. However, the lactic acid production is closely dependant on the price and availability of the sugar source for fermentation. The production of sugars is therefore at the moment in competition with food for land, although quantities dedicated to material applications are still small. The future will bring sustainable solutions using by-products or residues as a cheap carbon source for PLA production integrated into biorefinery systems.

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9

Biobased Composites and Applications

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Abstract

Biobased reinforced composites are low cost, low density, and have high specific properties along with inherent biodegradability and non-abrasive characteristics. These materials can be effectively utilized for high performance engineering applications in various sectors such as the automotive and aerospace industries, and for building and construction. The present chapter encompasses a critical review on the biobased composites, their characteristics and applications. Various experimental findings based on mechanical, dynamic mechanical, and thermal properties of biobased composites of sisal fiber and polypropylene are also discussed. The processability of these composites and their flow behavioral pattern has been presented. Newer innovations in the area of biobased hybrid composites are also discussed, highlighting the mechanical properties, degradation and flame retardency of Banana/Glass fiber PP hybrid composites. Furthermore, a brief idea on various types of applications of these composites has also been provided.

Keywords: Biobased, composites, MAPP, hybrid, flammability

9.1 Introduction

Low cost biofibers such as jute, sisal, hemp, flax, ramie, banana, coir, etc., have received considerable attention in the recent years. These materials have successfully replaced the synthetic fibers; glass in particular and other mineral fillers for fabrication of biobased composites used for engineering applications in various sectors such as aerospace, automobile, electronics, packaging, construction, etc.

Biofibers can be effectively reinforced within the polymeric matrices in different ways to achieve desired properties such as strength, stiffness, low density, and sound damping, along with eco-friendly characteristics and texture in the composites. The use of biofibers for various commercial applications originated back in the 1990's. Owing to low prices and the steadily rising performance of technical and standard plastics, the application of these fibers

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (225–266)

came to a near-halt. However, with the emerging trends for the preservation of natural resources and recycling technologies, a renewed interest concerning the use of natural materials with a focus on renewable raw materials has been generated [1]. Use of jute fibers as reinforcement for composites has gained popularity in recent years. Pipes, pultruded profiles, and panels with polyester matrices have been effectively fabricated employing these fibers [2]. Jute reinforced polyester resins have been extensively used as construction materials for buildings (e.g. Madras-House,1978) [3] and grain elevators.

Today, a renaissance in the use of biofibers as reinforcement in technical applications is taking place primarily in the automobile and packaging industries. Short biofiber reinforced composites of thermoplastic materials have emerged as a major class of structural materials in various advanced areas. These materials offer a unique combination of high strength to weight ratio, better dimensional stability, and heat and environmental resistance, that is comparable to, or better than, many conventional materials. Cambridge Industries, a giant in automotive composites, has been making flax/PP composites for Freightliner Century COE C-2 heavy trucks. The door panels in the Mercedes have also been successfully made from plastics reinforced with flax fibers. Canadian companies are also using flax fibers in a polypropylene matrix to create moldable material to form the rear shelf panel of the 2000 Chevrolet impala. The biofibers reinforced parts went into series production in 2004 and resulting in "potential lower cost and weight". Life cycle analysis of these components also shows that abaca (banana) fiber reduces primary energy use by 10%. Similar successful components have also been produced by Daimler Chrysler using biofibers such as jute, sisal, flax hemp, and coconut in vehicle interiors for several years. Rieter Automotive has replaced most of the glass fiber with abaca natural fiber as reinforcement in the spare wheel pan cover of the Mercedes A-class (W169) car. The use of flax fibers in car disc-brakes to replace asbestos-fibers is yet another example of this type of application [4].

This chapter will focus on the application of these biofibers in the area of biobased composites. Also, a clear presentation of various examples of biobased composites, their properties and characterization studies are reported in the discussion.

9.2 Biofibers: Opportunities and Limitations

In general, biofibers or lignocellulosic natural fibers, can be broadly classified into two categories, particulates and fibers. Particulates have an aspect ratio of approximately one, wherein no significant strengthening is expected although the elastic modulus and some other properties may be improved. Wood flour, ground rice hulls, ground corn cob, etc., fall under this category. Fibers are considered to be short when the aspect ratios vary between that of the particulate and continuous fibers. Wood fibers are the most widely used short fibers, but they can also be obtained from agro-bases from different parts of the plant such as bast (jute, abaca, flax, hemp, kenaf), leaf (pineapple, sisal, screw pine),

seed or fruit fiber (coir, cotton, oil palm), grasses and reeds (bamboo, sugarcane), etc. These fibers come from the xylem of angiosperm (hardwood) and gymnosperm (softwood) trees. Examples include maple, yellow poplar, and spruce. The potential fibers named above are separated from the original plant in several ways such as retting, scrapping, and pulping. Biofibers generally consist of helically wound cellulose microfibrils in an amorphous matrix of lignin and hemicellulose that run along the length of the fiber. The properties of these fibers are very difficult to measure with a considerable number of fibers (between 500 and 4000) needing to be tested to obtain statistically significant mean values ($p<0.05$). These properties are also strongly influenced by many factors, particularly chemical structure, which differ between different parts of a plant as well as between different plants. However, unlike brittle fibers, such as glass and carbon fibers, cellulose fibers are flexible and do not fracture when processed over sharp curvatures. This enables the fibers to maintain the desired aspect ratio for good performance. Their non-abrasive nature permits a high volume fraction of filling during processing, and this results in high mechanical properties without the usual machine-wear problems associated with synthetic fibers, especially glass and ceramic. Biofibers are also non-toxic [5–7], easy to handle, and do not have the health problems associated with glass fibers that can cause skin irritations and respiratory diseases when the fibrous dust is inhaled [8]. They offer a high ability for surface modification, are economical, require low amounts of energy for processing, and are biodegradable. In terms of socio-economic issues, the use of biofibers as the source of raw materials is beneficial because it generates an economic development opportunity for non-food farm products in rural areas. Because these advantages are beneficial, they are not likely to be ignored by the plastics industry for use in the automotive, building, and appliance sectors, along with other applications. A comparative account of various biofiber vis-à-vis conventional synthetic fibers is shown in Table 9.1 [9].

Despite the advantages mentioned above, the use of biofibers in thermoplastic matrices has not been extensive. Possible reasons that contribute to unsatisfactory final properties of the biobased composite include: (i) Limited thermal stability [9–14] at typical melt processing temperature of about 200°C. This limits the type of thermoplastic that can be used with the fibers [9–10], (ii) poor dispersion characteristics in the non-polar, olefin thermoplastic melt due to strong hydrogen forces between the fibers [15–16], (iii) limited compatibility with many thermoplastic matrices [17–18] due to their highly hydrophilic character [19], resulting in poor mechanical properties of the composites produced; (iv) high moisture absorption of the fibers [20–21] that can affect the dimensional stability of the composite [22] and the interface bond strength; and [23] high biodegradability when exposed to the environment. This limits the service life of composites, particularly in outdoor applications. There are many published reports on the potential use and limitation of cellulosic biofibers as reinforcement in thermoplastics. These studies show that the problems mentioned above are common, independent of the type and origin of the fiber employed [24–25]. Other factors that may hamper increased use of cellulose

Table 9.1 Comparative properties of biofibers and conventional man-made fibers [9].

Fiber Type	Density (g/cm^3)	Diameter (μm)	Tensile Strength (MPa)	Young's Modulus (GPa)	Elongation @break (%)
Jute	1.3–1.45	20–200	393–773	13–26.5	7–8
Flax	1.5	–	345–1100	27.6	2.7–3.2
Hemp	–	–	690	–	1.6
Ramie	1.5	–	400–938	61.4–128	1.2–3.8
Sisal	1.45	50–200	468–640	9.4–22	3.7
PALF	–	20–80	413–1627	34.5–82.51	1.6
Cotton	1.5–1.6	–	287–800	5.5–12.6	7–8
Coir	1.15	100–450	131–175	4–6	15–40
E-Glass	2.5	–	2000–3500	70	2.5
S-Glass	2.5	–	4570	86	2.8
Aramid	1.4	–	3000–3150	63–67	3.3–3.7
Carbon	1.7	–	4000	230–240	1.4–1.8

fibers in plastics are the problems and costs associated with the collection and storage, which are not yet mechanized and standardized to produce fibers of high and uniform quality [26–27].

9.2.1 Chemical Composition of Biofibers

Biofibers, as discussed in the previous section, are generally lignocellulosics, consisting of helically wound cellulose microfibrils in an amorphous matrix of lignin and hemicellulose. Cellulose molecules are laid down in microfibrils in which there is an extensive hydrogen bonding between cellulose chains producing a strong crystalline structure. In the wood cell wall, the microfibril is reported to be ovoid or almost square in cross section and 3–4 nm in diameter [28]. However, even though it is difficult to precisely estimate the microfibril length, values of several micrometers occur. The aspect ratio of individual microfibril is therefore high. The microfibrils in bast fibers from flax, jute and hemp are larger than those commonly found in wood. There is currently much debate as to the precise nature of microfibrils; however, it is clear that they are inherently very strong, with tensile strength superior to steel [29]. The fact that plant fibers in general are weaker reflects weaknesses in other domains of the cell walls. The isolation and utilization of microfibrils themselves in composites is an attractive thought that has received considerable attention [30].

The secondary wall found in wood cells is composed of two or three layers, known as S1, S2, and S3, respectively. In each of these layers, the cellulose microfibrils are "spirally-wound" at a different angle to the major axis of the tracheid. This variation in microfibril angle imparts strength to the fiber structure in a variety of directions. Within the bast or schlerenchyma cells found in flax, hemp, jute, and kenaf, the secondary wall is less thick than that of wood, but contains layers of similarly spirally-wound microfibrils embedded in a hemicellulose and pectin-rich matrix. This "composite structure" imparts potentially high strength to regions of the cell wall. Figures 9.1 and 9.2 show a schematic representation of flax fiber and a section of an elementary fiber with its fibrillar structure in its secondary cell wall [31].

Hemicelluloses cover the surface of the microfibrils, hydrogen bonding to outer cellulose chains. They are a group of heteropolysaccharides occurring within plant cells and are essentially "copolymers" of a number of sugars, most commonly glucose, mannose, xylose, galactose, and arabinose. Hemicelluloses

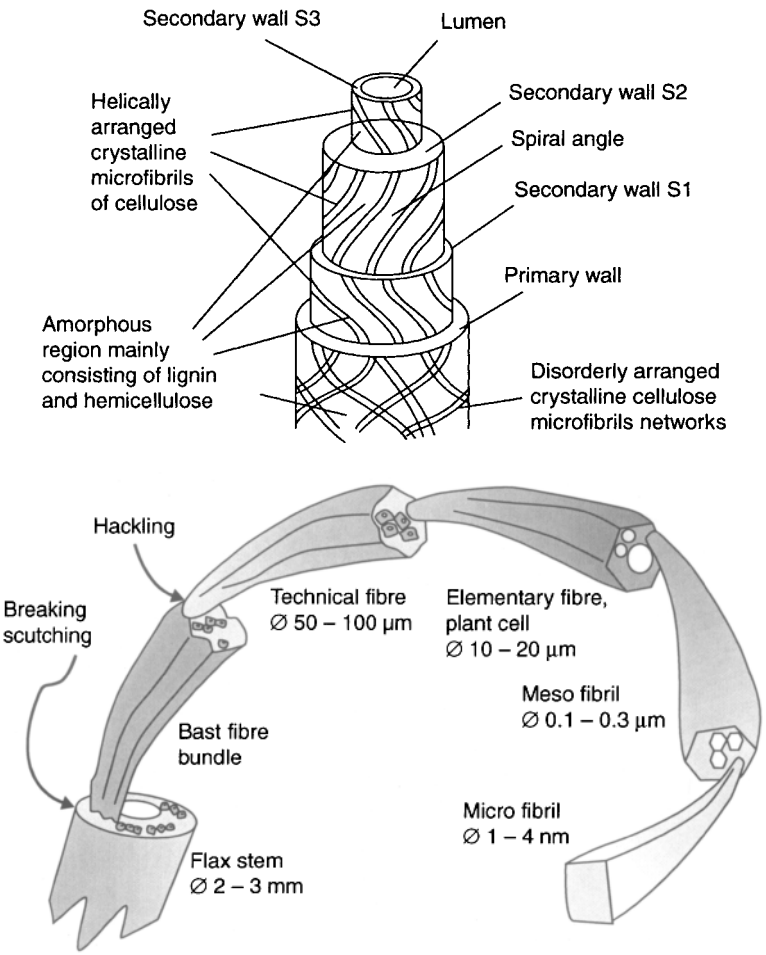


Figure 9.1 Schematic representation of flax fiber from stem to microfibril [9].

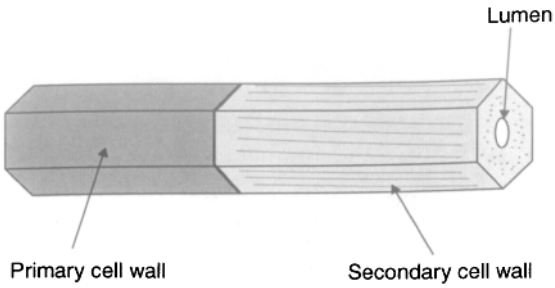


Figure 9.2 Schematic representation of a section of the elementary fiber with its fibrillar structure in the secondary cell wall [9].

are of lower molecular weight than cellulose, are occasionally branched, and invariably much less ordered than cellulose as a consequence of their more heterogeneous structures.

Hemicelluloses are very hydrophilic polymers and are largely responsible for the water sorption behavior exhibited by plant fibers (along with pectins when present). In many plant and wood species, the hemicelluloses tend to interface between the cellulose and the lignin (Shimizu, 1991) [32].

The third component of wood and plant cell walls is lignin which is generally regarded as an adhesive within the cell wall and is the final polymer to be laid down during cell development. Lignin is essentially a disordered, polyaromatic, and cross-linked polymer arising from the free radical polymerization of two or three monomers structurally related to phenyl-propane. The common monomers are generally alcohols, trans-coniferyl alcohol and trans-synapyl alcohol. Many non-wood lignins also contain units resulting from the co-polymerization of a third monomer trans-p-coumaryl alcohol (Sakakibara, 1991) [33]. Free radical coupling of the lignin monomers gives rise to a very condensed, reticulated, and cross-linked structure. The lignin matrix is therefore analogous to a thermoset polymer in conventional composite terminology. The lignin polymer is laid down between the hemicellulose zones surrounding microfibrils, conferring rigidity, a degree of hydrophobicity, and decay resistance to the cell wall.

In most non-wood fiber cells, pectin is a major matrix component within the cell wall. Pectins are polysaccharides and can have complex structures; they can be branched. The main chain is a polymer of (1–4)-α-D-glucuronic acid, in which the acid groups are partially esterified with methanol. At frequent intervals, residues of the sugar, rhamnose, are included in the main chain and side chains rich in arabinose and galactose sugars are attached to the rhamnose residues. In addition, acid groups between adjacent pectin chains are often cross-linked by calcium ions. This confers a degree of structural integrity and rigidity to regions of the cell wall rich in pectin. Pectin is an important component of non-wood fibers, particularly the important bast fibers from flax, hemp, and jute.

Ray [34], in his study on the influence of lignin content on the mechanical behavior of jute, found a gradual decrease in both the strength and stiffness

of the fiber with lignin removal. The extensibility of the fiber was also found to follow the same trend [35]. Similar experiments, which were carried out on sugarcane fiber [36] provided additional evidence of the significant contribution of lignin to fiber strength. The most efficient cellulose fibers are those with high cellulose content coupled with a low microfibril angle [37], such as jute that has a cellulose content of more than 60% and a microfibril angle in the range of 7–12^0 [38] to the fiber axis. Data on the chemical composition and microfibril angle, as well as their physical properties, is critical in order to dictate the specific use of a certain fiber, and this has been reported by many investigators in publications. The data, however, is incomplete and needs to be expanded to include other potential fiber sources. Other factors that may affect the fiber properties are maturity [39], separating process, microscopic and molecular defects such as pits and nodes [40–41], and type of soil and environmental conditions under which they were grown [42]. The chemical composition of different types of natural fibers is shown in Table 9.2 [9].

Table 9.2 Chemical composition of biofibers [9].

Fiber Type	Cellulose (Wt %)	Hemicellulose (Wt %)	Lignin (Wt %)	Pectin (Wt %)
Abaca	61–64	21	12	0.8
Bagasse	32–48	21	19.9–24	10
Banana	60–65	6–19	5–10	3–5
Bamboo	26–43	15–26	21–31	–
Coir	46	0.3	45	4
Cotton	82–96	2–6	0.5–1	5–7
Flax	60–81	14–19	2–3	0.9
Hemp	70–92	18–22	3–5	0.9
Jute	51–84	12–20	5–13	0.2
Kapok	13–16	–	–	–
Kenaf	44–57	21	15–19	2
Phormium	67	30	11	–
Pineapple	80–81	16–19	4.6–12	2–3
Ramie	68–76	13–15	0.6–1	1.9–2
Sisal	43–78	10–13	4–12	0.8–2
Wood	45–50	23–30	27	2–2.5
Henequen	77.6	4–8	13	–

9.2.2 Surface Modification and Characterization of Biofibers

Almost all natural organic-fibers and particularly biofibers, also known as plant-derived fibers, are hydrophilic in nature, mainly as a consequence of their chemical structures. Plant fibers contain polysaccharides, of which two types are very hydrophilic: the hemicelluloses and the pectins. Hydroxyl and carboxylic acid groups located on these branched heteropolysaccharides are active sites for the sorption of water (Avramidis, 1997) [43]. The cellulose component is also hydroxyl group rich, but as a consequence of its linearity and high crystallinity, little water can be accommodated within the microfibrils, so the polymer is essentially less hydrophilic than may be expected. However, free, non-H-bonding OH groups on the microfibril surfaces are available for sorption. It should also be remembered that plant fibers are produced within dynamic, living, aqueous environments and, until the cell wall lignification stage of growth, are designed for their optimal performance in the living plant in water-rich environments.

The consequences of this hydrophilic nature of plant fibers for matrix polymer reinforcement can be profound. Many of the common matrix polymers in composites ranging from polyolefins to polyesters and epoxies are largely hydrophobic in nature. Thermosets such as phenolformaldehyde and related polymers are less hydrophobic in nature and are therefore less problematic. In general, there is a poor surface wetting of plant fibers by most thermoplastic polymers commonly used in composites. This can lead to the formation of ineffective interfaces between the fiber and matrix phases, with the consequent problems such as poor stress transfer, small void spaces, and de-bonding in the resulting composite materials. Indeed, initial problems encountered in "wetting out" plant fiber mats and rovings with polyolefins have led to an aversion to these materials within parts of the industry. Furthermore, dimensional instability in conditions of varying humidity contributes to the hydrophilic nature of cell wall polysaccharides and leads to a tendency of plant fibers to swell and shrink as they gain or lose water, especially in composites where the relative proportion of fibers is high. In such cases, the swelling of fibers in conditions of high humidity or direct exposure to liquid water causes stress within the surrounding matrix and leads to composite damage and eventual failure [44].

The aforementioned problems can be largely averted by fiber surface sizing, coating, or chemical modification. Surface coating is a simple but expensive solution. A more satisfactory approach is the transformation of surface hydroxyl groups by chemical modification. The polysaccharide and lignin components of plant fiber cell walls and surfaces are chemically reactive; indeed, this is reflected in the water sorption behavior of the materials. This inherent reactivity can be harnessed and hydrophobic monomers, oligomers, or even polymer chains can be covalently attached to the fiber surface. Such reactive modifiers can be loosely described as compatibilizers. Simple chemical modification of wood and plant fibers has received much attention over the past 20 years or so [45–47]. Techniques such as acetylation, wherein surface hydroxyl groups are reacted with acetic anhydride to produce more hydrophobic acetyl

ester groups, have been proven moderately successful in improving the non-specific compatibility of plant fiber with matrix polymers and the dimensional stability of the fibers themselves [48]. However, this has broadened out over recent years, and a number of potentially effective modification systems and compatibilizers have been examined and developed.

9.2.2.1 *Physical Modification*

Physical methods involve surface fibrillation, electric discharge (Corona, cold plasma) [49–50], etc. Physical treatments change structural and surface properties of the fiber and thereby influence the mechanical bonding with the matrix. Surface modification by discharge treatment such as low temperature plasma, sputtering, and corona discharge is of great interest in relation to the improvement in functional properties of lignocellulosic fibers. Low temperature plasma treatment causes mainly chemical implantation, etching, polymerization, free radical formation, and crystallization; whereas sputter etching chiefly brings about physical changes such as surface roughness, which leads to an increase in adhesion and decreased light technique to improve the surface characteristics of the fiber and polymeric materials by utilizing the ingredients such as electron, ion, radical and excited molecules produced by electrical discharge. Low temperature plasma can be generated under atmospheric pressure in the presence of helium [50].

The action of this plasma involves abstraction of protons and creation of unstable radicals that convert functional groups such as alcohols, aldehydes, ketones, and carboxylic acids. Yuan *et al.* [51] have investigated the effect of plasma treatment in enhancing the performance of wood fiber reinforced polypropylene composites. Argon and air-plasma treatments were used to modify the surface of radiata pine wood fibers to improve the performance in the composites under various static and dynamic conditions. An improvement in the tensile strength and modulus and dynamic mechanical properties of wood fiber-PP composites has been reported. Electrical discharge methods are used for cellulose fiber modification to decrease the melt viscosity of cellulose polyethylene composites [7] and to improve the mechanical properties. Corona treatment is one of the most interesting techniques for surface oxidation activation. It changes the surface energy of the cellulosic fibers, which in turn affects the melt viscosity of composites [49]. Mechanical and rheological properties of cellulose-PP composites subjected to corona treatment were reported by Sapieha *et al.* [52–53]. Corona treatment modifies the surface composition, thereby improving the surface properties of the composite components.

9.2.2.2 *Chemical Modification*

Chemical modification is generally taken to mean a process involving the creation of a chemical bond (usually covalent) between a surface cell wall polymer and an introduced reagent to form a novel adduct. This can be done through several approaches, including plasma activation and graft polymerization with vinyl monomers, which are very well described in literature. The primary

drawback of using biofibers is their limited thermal stability with noticeable degradation occurring as the melt processing temperature approaches 200°C [10]. This excludes some manufacturing processes and limits the type of thermoplastic that can be used on such low-temperature polymers as polypropylene, polystyrene, and polyethylene [11]. Higher processing temperatures that reduce melt viscosity and facilitate good mixing, however, are possible, but only for short periods. If degradation occurs, biofibers can be responsible for the formation of tar-like products and pyrolysis acids that may have various damaging effects both on the processing equipment and the composite properties [54].

9.2.3 Physical and Mechanical Properties of Biofibers

The diameter of biofibers normally varies in the range of $0.015 \times 10^4 - 0.05 \times 10^4$ μm. The densities of biofibers are in the range of 1.25 – 1.55 g/cm^3. The mechanical properties of biofibers have already been summarized in Table 9.1. Based on the results reported in the table, it could be observed that bast and leaf fibers are the strongest among the biofibers with a high tensile strength and modulus of elasticity. However, owing to their low extensibility, bast and leaf fibers have poor toughness properties compared to seed fibers such as oil palm fiber and coir fibers. According to Sreekala *et al.* [55] and Bledzki *et al.* [56], the mechanical properties of biofibers depend on the fibrillar structure, spiral angle of the micro-fibrils, and the cellulose content.

The relationship between the strength of the biofibers with the microfibrillar angle and cellulose content is given in the following equation:

$$\sigma = -334.005 - 2.830\theta + 12.22W \tag{9.1}$$

where σ is the fiber strength, θ is the microfibrillar angle and W is the cellulose content.

Furthermore, the elongation at break can be correlated with the microfibrillar angle θ based on the equation shown below:

$$\varepsilon = -2.78 + 7.28 \times 10^{-2}\,\theta + 7.7 \times 10^{-3}\,\theta \tag{9.2}$$

According to Bledzki *et al.* [56], high cellulose content and smaller spiral angle would result in the increase of the biofiber strength. On the other hand, *Lilholt et al.* [29], stressed that the strength of biofibers are strongly affected by various factors such as the structure of the fibers, orientation of molecular chains, imperfection or defects in the fibers, and the degree of polymerization. The regions with non-crystalline structure such as the amorphous regions in the cell wall of the biofibers are considered as weak points owing to the low number of chains per cross sectional area which are unable to withstand the stresses effectively. The orientation of the crystalline components influences the strength of the fibers. High strength fibers are normally achieved with alignments within about 50% of perfect orientation. The imperfection in the fiber

structure such as the presence of fiber bundles which results in non-uniform stress distribution, traces of oil especially found on oil palm fibers, and cracks on the fiber surface reduce the strength of the biofibers. The strength of highly oriented biofibers is affected by the degree of polymerization. Lilholt and Lawther [29] also explained that the strength of the fibers is inversely proportional to the degree of polymerization.

9.3 Biobased Composites: An Overview

A composite involves the marriage between a fiber and a polymer matrix. The constituents retain their identities, as they do not dissolve or merge completely into one another although they act in concert. Normally, the components can be physically identified and exhibit an interface between one another. These composite materials made from two or more constituent materials with significantly different physical or chemical properties, remain separate and distinct on a macroscopic level within the finished structure. The key advantage of working with composite materials is the opportunity to integrate material properties and design and manufacture techniques so that the end product is a completed structure optimized for both performance and economic stand points. Biobased composites involve the combination of a biofiber with a thermoplastic or thermoset matrix. These materials show environmentally friendly characteristics while enhancing the mechanical strength and thermal stability in the base matrix.

The reinforcement caused by short biofibers in a biobased composite is primarily governed by the following parameters: i) Fiber dispersion, ii) fiber-matrix adhesion, iii) fiber aspect ratio, iv) fiber orientation, and v) fiber volume fraction [3–4]. Studies to understand the influence of these factors on biobased composites have been carried out and reported by many investigators such as Felix [16] and Raj and Kokta [20]. The authors investigated the influence of various dispersing aids (stearic acid and mineral oil) and a coupling agent (maleated ethylene) in biofiber reinforced polypropylene composites. Tensile strength and modulus of the composites were found to increase with fiber content when either strearic acid or mineral oil (1% by weight of fiber) were added as processing aids during the compounding. The properties also were found to be affected by the amount of processing aid used. Maximum increase in the properties was observed when the processing aid was added in 1% concentration (by weight of fiber). A further increase in the amount of processing aid caused the properties to decline dramatically. Stearic acid was found to perform better in improving the fiber dispersion compared to mineral oil. Good fiber dispersion is generally the ultimate objective of any mixing process [18]. Different mixing techniques, however, do not produce biobased composites with the same degree of fiber dispersion. Woodhams *et al.* [57] used a thermokinetic mixer to mix cellulose fibers with thermoplastics and found the technique effective in dispersing the cellulose fiber within thermoplastic matrices [52, 56]. The effectiveness of the technique was then confirmed by

Sanadi *et al.* [58]. Lilholt *et al.* [29] investigated the effect of several processing techniques on the properties of polypropylene composites reinforced with short sisal fibers. The equipment used involved a cold mixer, press, injector, two-roll mill, and twin-screw extruder. The sisal fibers were pretreated with silane prior to processing to ensure good compatibility with the matrix. From the study, they concluded that processing techniques and routes can greatly influence composite properties. Both were also found to produce a great variation in the dimension and dispersion of the sisal fibers within the composites studied. The best processing method involved a twin-screw extruder. In case of biobased composites, weak adhesion may result from poor dispersion and incompatibility between the hydrophilic biofibers and the hydrophobic polymer. A direct measure of adhesion between the biofibers and thermoplastics is bonding strength. Several reports pertaining to the use of maleated coupling agents (MAPP) as a coupling agent in biobased composites have been extensively investigated by various workers. Interactions between the anhydride groups of maleated coupling agents and the hydroxyl groups of biofibers can overcome the incompatibility problem to increase tensile and flexural strength of biofiber thermoplastic composites. The composites prepared using Epolene G 3015, a medium molecular weight, and medium acid number maleated coupler displayed optimum performance at a concentration of 3 wt %. Studies reported by Mohanty *et al.* [59] for sisal reinforced PP composites showed an increase in tensile strength to the tune of 40% and flexural and impact strength to 31%, and 58% in the case of sisal PP composites at a fiber loading of 30% and MAPP (G 3015) concentration of 1%, as compared to the untreated fiber composites. Furthermore, SEM investigations confirmed efficient interfacial adhesion between the fibers and PP matrix. MAPP treated fiber reinforced composites manifested lesser fiber pull outs as evident from Figures 9.3a and b. The chemical bonding between the anhydride and the hydroxyl groups caused a better stress transfer from the matrix into the fibers, leading to a higher tensile strength.

Gatenholm *et al.* [23] studied the nature of adhesion in the composites of modified cellulose fibers and polypropylene. Biofibers were surface-modified

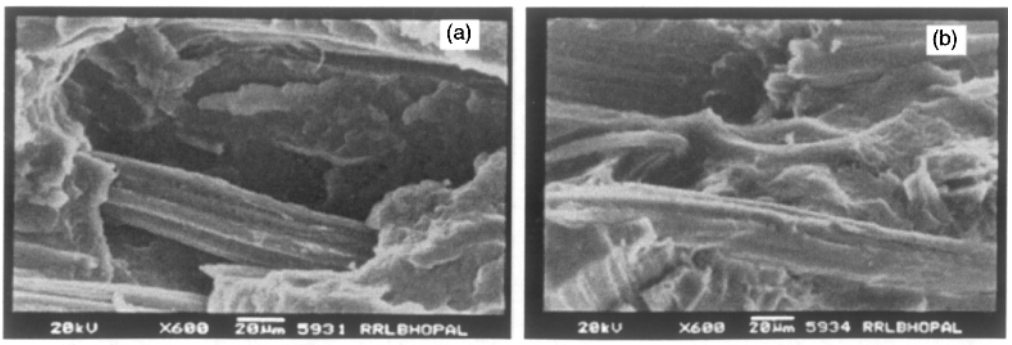


Figure 9.3 SEM micrograph (a) untreated sample at 45% fiber loading and (b) treated sample at 45% fiber loading [59].

with polypropylene maleic-anhydride copolymer and characterized by contact angle measurement, ESCA, FTIR, and SEM techniques. Composites reinforced with surface-modified biofibers showed significantly improved mechanical properties compared to composites with untreated biofibers. This was due to improved fiber wetting, dispersion, and fiber-matrix adhesion as confirmed from SEM investigations. Interfacial interactions involved were covalent and hydrogen bonds that formed across the fiber-matrix interface.

The surface energy of fibers is closely related to the hydrophilicity of the fiber [6]. Some investigations are concerned with methods to decrease the hydrophilicity. The modification of wood-cellulose fibers with stearic acid [10] hydrophobizes those fibers and improves their dispersion in polypropylene. As can be observed in jute reinforced polyester composites, treatment with polyvinylacetate increases the mechanical properties [10] and moisture repellence [60]. Several similar investigations on biobased composites based on thermoplastic and thermoset matrices have been reported by several authors [61–63]. Some of the major findings based on the authors own experimental investigations are discussed in the following sections.

9.3.1 Biobased Composites of Sisal Fiber Reinforced Polypropylene

Over the last few decades, there have been several reports pertaining to the use of sisal fibers as reinforcements in polymer matrices [64–67]. The viscoelastic properties and rheological properties of LDPE filled with short sisal fiber as a function of fiber length, fiber content and fiber orientation were investigated by Joseph *et al.* [68]. A maximum storage modulus in the longitudinally oriented composites was reported. Furthermore, it was found that critical fiber length is necessary to obtain maximum dynamic moduli. It was also found that in all the cases, storage moduli (E′) and loss moduli (E″) decreases with temperature and increases with fiber loading. Preparation of sisal fiber reinforced PP composites (SFRP) through melt mixing and solution mixing methods were reported on by Joseph *et al.* [68]. A mixing time of 10 minutes with a rotor speed of 50 rpm and a mixing temperature of 170°C were optimized. The tensile properties were evaluated as a function of fiber length, fiber loading, and fiber orientation. The tensile properties of melt-mixed and solution-mixed composites were compared, and melt-mixed composites were reported to show improved performance over solution-mixed composites. LeThi *et al.* [69] have studied the mechanical properties of sisal fiber reinforced polypropylene composites prepared by a reactive extrusion. It was reported that the grafting of the fibers by MAPP enhanced both the impact strength and the breaking stress in the composites. The influence of fiber length, fiber content, fiber orientation, and benzoylation on the tensile properties of the sisal/PS composites have been evaluated by Nair *et al.* [67]. Similar studies on sisal fiber based PE composites, and the effect of fiber surface treatment on the fiber matrix bond strength have been investigated by Valadez-Gonzalez *et al.* [70]. They observed that the interfacial shear strength between sisal fibers and polyethylene matrix

was improved by the chemical modification of the fiber surface with silane. The influence of short glass fiber addition on the mechanical properties of short sisal fiber reinforced LDPE composites have been reported on by Kalaprasad *et al.* [65]. They observed that the addition of a small volume fraction of approximately 0.03% of short glass fiber into the above system enhanced the tensile strength of longitudinally oriented composites by more than 80%. It was also observed that water absorption tendency of the composite decreases with the process of hybridization. However, despite several advantages, the use of sisal fibers as reinforcements in thermoplastic composites has been limited. The performance characteristics of sisal fiber reinforced composites varies greatly depending on the fiber properties such as maturity, separating process, microscopic and molecular defects such as pits and nodes, and the type of soil and weather conditions under which they were grown.

9.3.1.1 *Mechanical and Dynamic Mechanical Properties of Sisal PP Composites*

The mechanical properties of virgin PP, treated and untreated sisal PP composites, as a function of fiber loading, are presented in Table 9.3 [71]. It is evident that the mechanical properties of the composites increased linearly with the increase in fiber loading from 6.8 to 21%. Tensile strength increased to about 64%, whereas flexural and impact strengths increased to about

Table 9.3 Mechanical properties of Sisal/PP biobased composites [71].

Fiber Vol%	Tensile Strength (MPa)	Tensile Modulus (MPa)	Impact Strength (J/m)
PP (virgin)	17.80±2.02	19.60±6.11	23.25±4.71
PP+sisal (6.3 vol%)	24.17±3.3	34.83±4.2	40.50±2.35
PP+sisal (10.3 vol%)	26.11±1.82	46.35±3.28	46.10±2.96
PP+sisal (21.0 vol%)	29.25±3.05	48.96±5.11	51.79±3.99
PP+sisal (31.0 vol%)	23.21±3.02	43.41±5.13	39.83±3.91
PP+sisal (21 vol%) + 0.3% MAPP	32.35±3.93	50.13±3.66	52.45±2.96
PP+sisal (21 vol%) + 0.5% MAPP	35.44±4.16	52.44±3.45	57.16±2.12
PP+sisal (21 vol%) + 1% MAPP	43.66±3.2	62.42±3.43	68.66±4.16
PP+sisal (21 vol%) + 2% MAPP	34.55±3.91	51.66±4.3	55.30±2.08

119 and 123%, respectively, as compared with the virgin polymer. This behavior is primarily attributed to the reinforcing effect of the fibers, leading to a uniform stress distribution from a continuous polymer matrix to a dispersed fiber phase. However, it was observed that with the increase in fiber loading from 21 to 31 volume percent, all the mechanical properties deteriorated. A decrease of nearly 26, 13.2, and 30% in tensile, flexural, and impact strengths, respectively, was noticed. This decrease in the mechanical properties at high fiber loading is probably due to incompatibility of the fibers within the matrix, which promoted micro-crack formation at the interface as well as non-uniform stress transfer due to fiber agglomeration in the matrix [71, 72]. On the other hand, the MAPP treated composites at 21% fiber loading exhibited improved mechanical properties in comparison to the untreated composites at the same volume percent of fiber content. The composite prepared using 1% MAPP concentration showed optimum mechanical strength. Tensile strength increased by 49%, while flexural and impact strengths increased by 30 and 58%, respectively, as compared with 30% for the untreated composite. This increase in the mechanical strength possibly occurred due to improved interfacial adhesion between the fibers and the matrix, due to the formation of ester linkage at the interface. The mechanical findings have been corroborated with morphological investigations depicted in Figure 9.4 [71]. In case of untreated composites

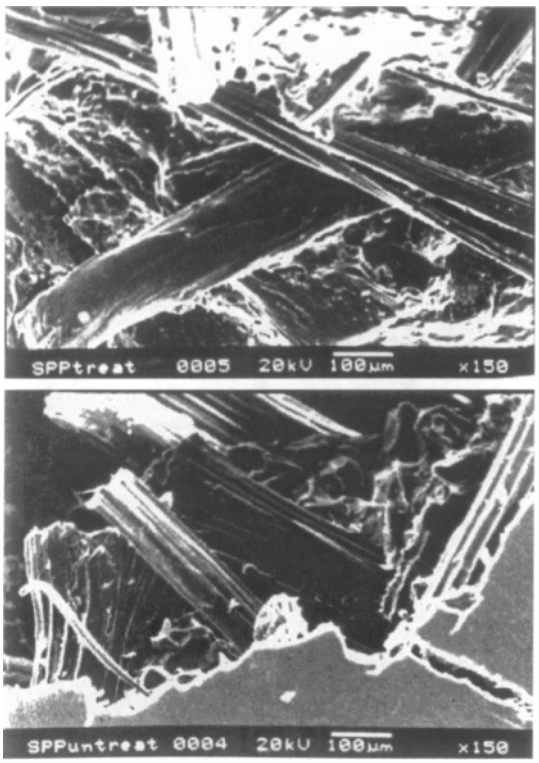


Figure 9.4 SEM micrographs of untreated sisal/PP composites at 30% fiber loading MAPP (1% concentration) treated [71].

(Figure 9.4, upper micrograph), well-defined holes of pulled out fibers could be observed. This is mainly due to weak interfacial interactions between the fibers and the matrix. On the contrary, with 1% MAPP treatment (Figure 9.4, lower micrograph), the scanning electron micrographs manifested improved adhesion. The fiber surface is well impregnated by a thin polymer layer, which reduced fiber pullouts and the gaps between the matrix and the fibers. In the same case, some gaps between the matrix and fibers were still noticed while the rest of the fibers seemed to be firmly bonded with the matrix. This is probably due to displacement of the fibers by the tensile forces [72, 73].

The dynamic mechanical analysis of virgin PP, untreated sisal/PP composites and MAPP treated sisal PP composites revealed an increase in the storage modulus (E′) in the PP matrix with the addition of fibers and MAPP (Figure 9.5a) [71]. The loss modulus displayed three relaxation peaks at -80°C (γ), 8°C (β), and 100°C (α), respectively. The temperature of β relaxation maximum corresponds to the Tg of the matrix, while the α relaxation peak is related to the slip mechanism in the crystallites. The γ relaxation peak is due to the

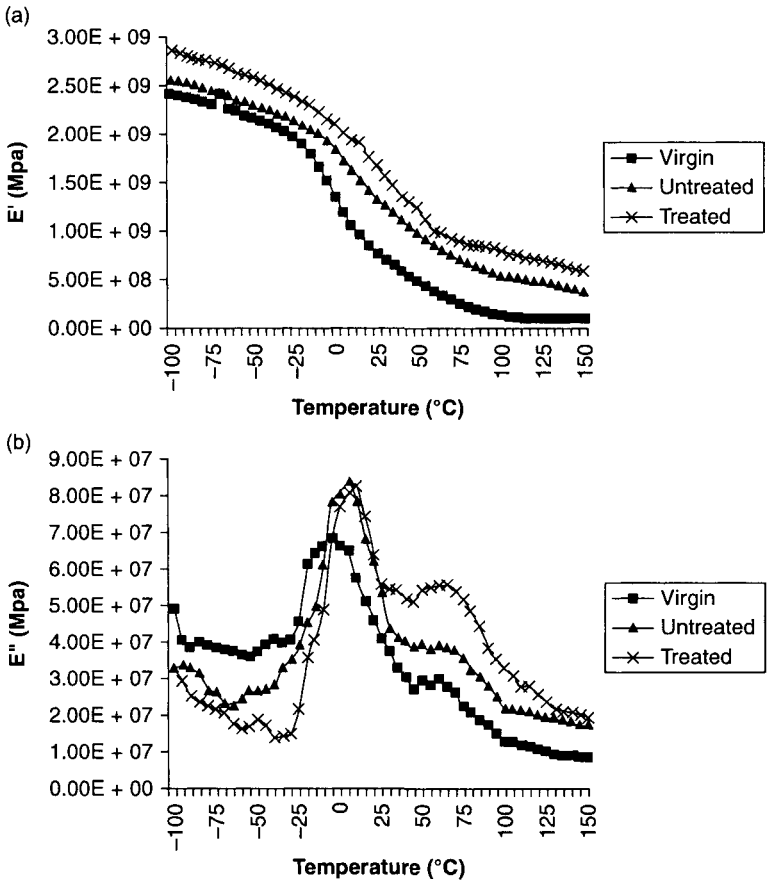


Figure 9.5 Variation of (a) storage modulus (b) loss modulus of virgin PP and untreated and treated sisal–PP composites as a function of temperature [71].

motion of small chain groups like methyl and methylene [73]. As evident from Figure 9.5b [71], in the case of virgin PP, the maxima of the peak at a temperature of –5°C is associated with the Tg of the matrix, whereas the high temperature peak at 55°C is probably related to the onset of melting of PP crystallites. In the composites prepared from 21% untreated fibers, the primary transition peak, that is, Tg, shifted to a comparatively higher temperature (5°C). This is primarily attributed to the immobilization of polymer molecules near the surface of sisal fibers due to molecular interactions caused by the latter. The E" values corresponding to the Tg in the untreated composites (8.39E+07 MPa) also increased considerably, to about 30%, in comparison to virgin PP (6.48E + 07 MPa). A similar shift of Tg to 10°C was also observed with the MAPP treated composites, which implied efficient fiber matrix interfacial adhesion. However, the loss modulus value (8.09E + 07 MPa) at this temperature showed a decrease to 4%, thereby indicating the presence of a genuine interface [29]. The high temperature α relaxation peak of virgin PP also showed a comparative increase, to about 60°C and 67°C in the untreated and treated composites, respectively. In the matrix polymer, however, there was virtually no sharp inflection point of this process. Conversely, the filled composites displayed an increase in peak height and broadening of the relaxation region. This behavior is probably due to inhibition of the relaxation process, resulting in the decrease in the mobility of polymer chains in the crystallites [73].

9.3.1.2 Thermal Properties of Sisal PP Composites

The thermogravimetric and differential thermogravimetric curves of virgin PP, sisal fiber, and untreated and treated composites are presented in Figure 9.6 [71]. In the case of sisal fiber (Figure 9.6b), the initial peak between 30 to 150°C indicates removal of moisture from the fiber with a temperature maximum of 57.7°C. The percentage of weight loss at this stage is about 6%. At 200°C and thereafter, the decomposition of the fiber takes place at a faster rate. As revealed from the DTG curve (Figure 9.7b), the primary decomposition temperature occurs at 380°C corresponding to a weight loss of about 72%. This is possibly due to thermal cleavage of the glycosidic linkage by transglycosylation and scission of C-O and C-C bonds, and loss of α cellulose from the fiber. A charred residue of carbonaceous products [25] was obtained above 592.1°C. The loss of hemicellulose from the sisal fiber occurs at 307.2°C, as revealed from the DTG curve. It is evident that the major decomposition range of hemicellulose and α cellulose lies between 207 to 592.1°C. The TGA/DTG curves of virgin PP, represented in Figures 9.6a and 9.7a, respectively, indicate that decomposition takes place at a temperature of 390°C, and nearly 100% decomposition occurred at 490°C. This temperature range was comparatively higher than those of the fibers. Step analysis of the PP-TG scan reveals 0% weight loss from 30 to 150°C. On the contrary, TGA/DTG curves reveal a comparatively higher thermal stability of the PP matrix with the incorporation of fibers. The minor decomposition peak observed at 410 and 408°C in the untreated (Figure 9.6c) and treated (Figure 9.6d) composites corresponds to

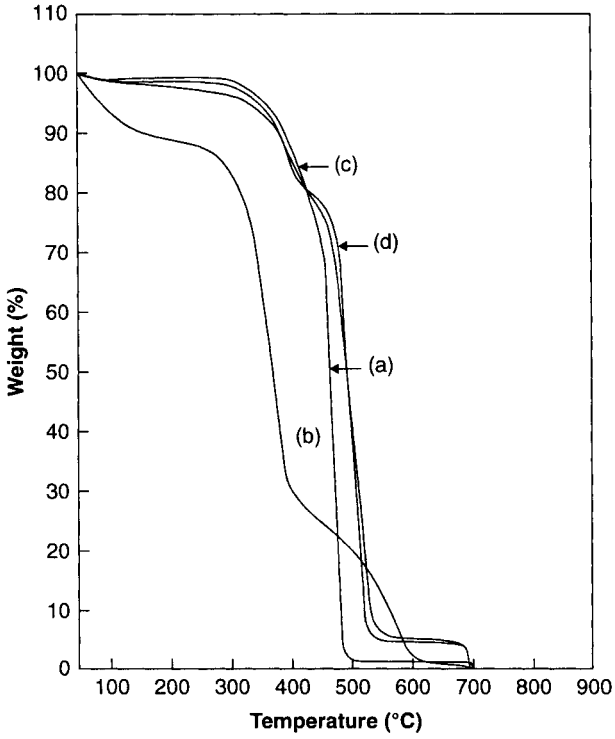


Figure 9.6 TGA of (a) virgin PP, (b) untreated sisal–PP composite, and (c) treated sisal–PP composite [71].

the degradation of PP. The major degradation at 510°C as revealed from the DTG curve (Figure 9.7c and 9.7d) is possibly due to degradation of dehydrocellulose. Comparing the weight loss at 400°C, approximately a weight loss of about 30% for the untreated fibers and 26.2% for the treated composite was noted. This shows a marginally higher thermal stability in the MAPP treated composite, thus confirming the presence of intermolecular bonding between the fibers and the matrix due to the formation of ester linkage [73].

9.3.1.3 Steady State Melt Rheology

The variation of steady state viscosity (η) as a function of shear rate ($\dot{\gamma}$) is represented in Figure 9.8 [74]. The viscosity data obtained from parallel plate and capillary rheometer were combined for investigating the behavior of the materials under variable shear rates. It is evident that the viscosity of the virgin polymer increased progressively with the increase in fiber loading from 10 to 30%. This is because in the filled systems, the fibers perturb the normal flow of the polymer and hinder the mobility of the chain segments in the direction of flow [75]. In the range of low shear rates, the fibers displayed larger reinforcing capabilities, which is primarily attributed to fiber–fiber interactions arising from weak structures made up by agglomerates of nonaligned fibers [74]. The melt flow curves depicted in Figure 9.8 reveals that all the systems show

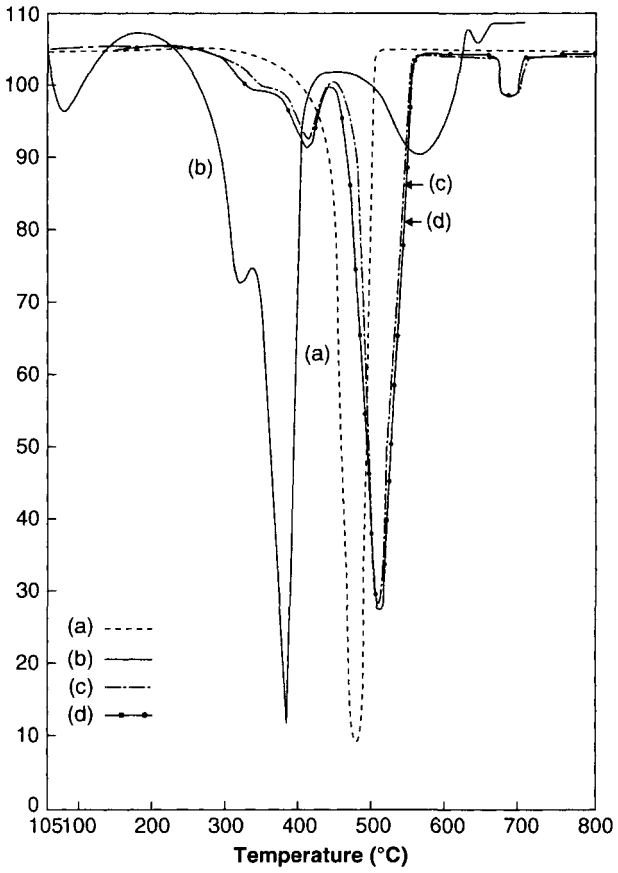


Figure 9.7 DTG of (a) virgin PP, (b) untreated sisal–PP composite, and (c) treated sisal–PP composite [71].

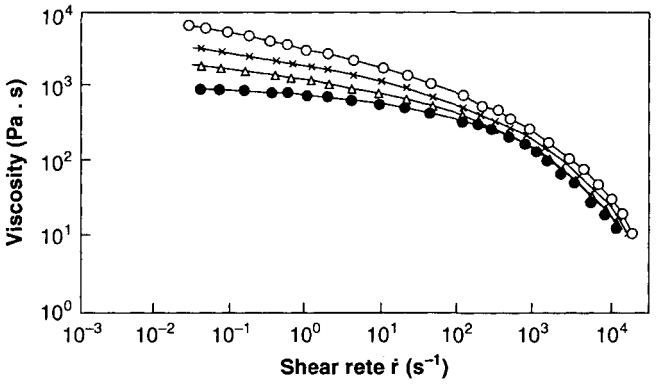


Figure 9.8 Effect of fiber loading on the steady state viscosity (•) virgin PP, (Δ) 10% PP/sisal, (×) 15% PP/sisal, (o) 30% PP/sisal [74].

non-Newtonian pseudoplastic behavior. In the range of high shear rates, shear-thinning behavior of the melt was observed and all the composites exhibited nearly the same viscosity. This phenomenon is probably due to alignment of the fibers at high shear rates along the tube axis thereby decreasing fiber to fiber collisions [75]. Goldsmith and Mason [76] have made similar investigations and have observed radial migration of filler particles towards the capillary axis during shear flow. Thus in a fiber-filled system the region close to the tube wall is virtually fiber free which results in close viscosity values of all the composites at high shear rates. The non-Newtonian pseudoplastic characteristics was further corroborated by fitting the curves obtained in the high shear rate regions, in the Ostwald-de-Waele or Power law equation:

$$\tau = K\dot{\gamma}^n \tag{9.3}$$

Where K is a constant related to the coefficient of viscosity and n is the flow behavior index. The values of K and n of PP and the composites at different weight percent of fiber loading, calculated by using linear regression analysis method, is shown in Table 9.4. As implied from the test results reported in the table, the flow behavior index n was less than one in all cases, which further corroborates non-Newtonian pseudoplastic character of the melt. However, the degree of flow behavior index (n) decreased with the incorporation of the fibers, thus indicating an increase in pseudoplasticity. Conversely, the K value increased in the composites revealing an increase in the viscosity of the virgin matrix. The effect of the addition of MAPP on the viscosity of PP/sisal composites is shown in Figure 9.9. Furthermore, a comparative account of the variation of η of the virgin matrix and the 30% untreated composite has also been included in the same figure to study the effect of surface modification. It is evident that the viscosity of the composites increased with the addition of MAPP. This behavior is primarily attributed to improved fiber-matrix interfacial adhesion. The reaction between the sisal fiber and MAPP is explained in the following reaction (Scheme 9.1) [74]. Additionally, the friction between the

Table 9.4 Effect of fiber loading and MAPE concentration on K and n values [74].

Sample Type	$K \times 10^2$	$n \times 10^{-1}$
PP Virgin	6.07	5.62
10% untreated	7.88	5.09
15% untreated	8.45	4.89
30% untreated	7.01×10^1	4.13
1% MAPP	8.34×10^1	3.96
2% MAPP	8.51×10^1	3.90

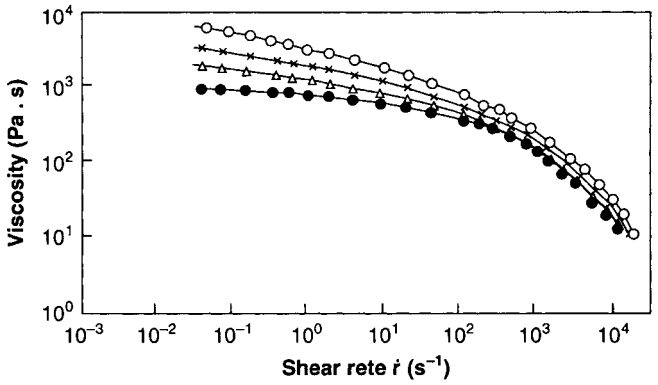
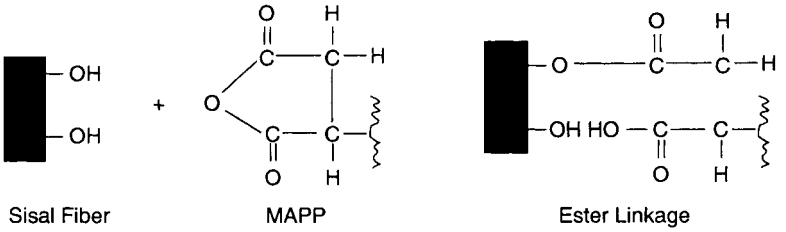


Figure 9.9 Effect of addition of MAPP on steady state viscosity (•) virgin PP, (Δ) 30% PP/sisal, (×) 1% MAPP, (o) 2% MAPP [74].



Scheme 9.1 Hypothetical model of esterification reaction between the hydroxyl groups of sisal fibers and anhydride rings of MAPP [74].

polymer and the fibers is also increased resulting in an increase in the viscosity of the composites. However, the flow curves of the treated composites also exhibited similar pseudoplastic and shear thinning characteristics. The values of K and n derived from the Ostwald-de-Waele equation showed an increase in the K value (Table 9.4) [74] with the addition of MAPP, thus confirming an increase in the viscosity of the melt. The degree of flow behavior index also increased, thus revealing an increase in pseudoplasticity of the treated composite melts [75].

The variation of die swell ratio De/D (where De and D are the diameters of the extrudates and die) of virgin PP, untreated and treated composites is represented in Table 9.5 [74]. It is evident that the swell ratio of the virgin matrix decreased with the incorporation of fibers. This is probably attributed to the distribution of fibers within the PP matrix which results in stress transfer from matrix to the fiber, thereby retarding the elastic recovery of the material [75]. Furthermore, the treated composites displayed a lesser die swell, thus confirming enhanced fiber-matrix adhesive strength. However, the swell ratio increased with the shear rate, which is probably due to the decrease of normal stress and elastic recovery at low shear rates.

Table 9.5 Die swell ratio of virgin PP, untreated and treated PP/sisal composites at 30% fiber loading [74].

Sample Type	Shear rate (s^{-1})	
	15	150
PP Virgin	1.21	1.38
30% untreated	1.20	1.36
2% MAPP	1.17	1.34

9.3.1.4 Extrudate Morphology

The SEM micrographs of the cross section of the extrudates in untreated and treated composites at 30% fiber loading is depicted in Figures 9.10a–d, respectively [74]. It is evident that at high shear rates of 150 s^{-1} the cross section of the extrudates of both untreated and treated composites revealed fairly efficient dispersion of the fibers within the matrix. The extrudate cross section also depicted the evidence of few unwetted fibers along with the presence of voids, which is probably due to incompatibility of the fibers within the matrix resulting in fiber agglomeration at higher loading of 30% (Figure 9.10a and b). Furthermore, the fractured surfaces of the cross section in both the composites are virtually identical, with relatively lesser fiber pullouts irrespective of the addition of MAPP (Figure 9.10 c and d). Since gross fiber pullout is viewed as evidence of poor fiber matrix adhesion, this system (both treated and untreated) appears to display favorable interaction between the matrix and the fiber phases. This behavior is probably at high shear rates, since the fibers are aligned along the flow directions uniformly. Moreover, the resistance to the motion of the fibers and polymer matrix is also reduced along with a reduction in viscosity of the polymer matrix. Similar facts have been substantiated in our experimental findings.

9.3.2 Innovations in Biobased Hybrid Composites

Hybrid composites are materials made by combining two or more different types of fibers in a common matrix [77]. Hybridization of two types of short fibers having different lengths and diameters offers some advantages over the use of either of the fibers alone in a single polymer matrix [78]. Hybridization allows designers to tailor the composite properties to the exact needs of the structure under consideration [79]. In most cases, the purpose of hybridization is to obtain a new material retaining the advantage of its constituents and hopefully overcoming some of their disadvantages. Another desired achievement is related to the cost, with one of the components being generally less expensive than the other.

Biofiber reinforced hybrid composites have been the major subject of research in recent years. Most published reports limited to the hybrid composite consist

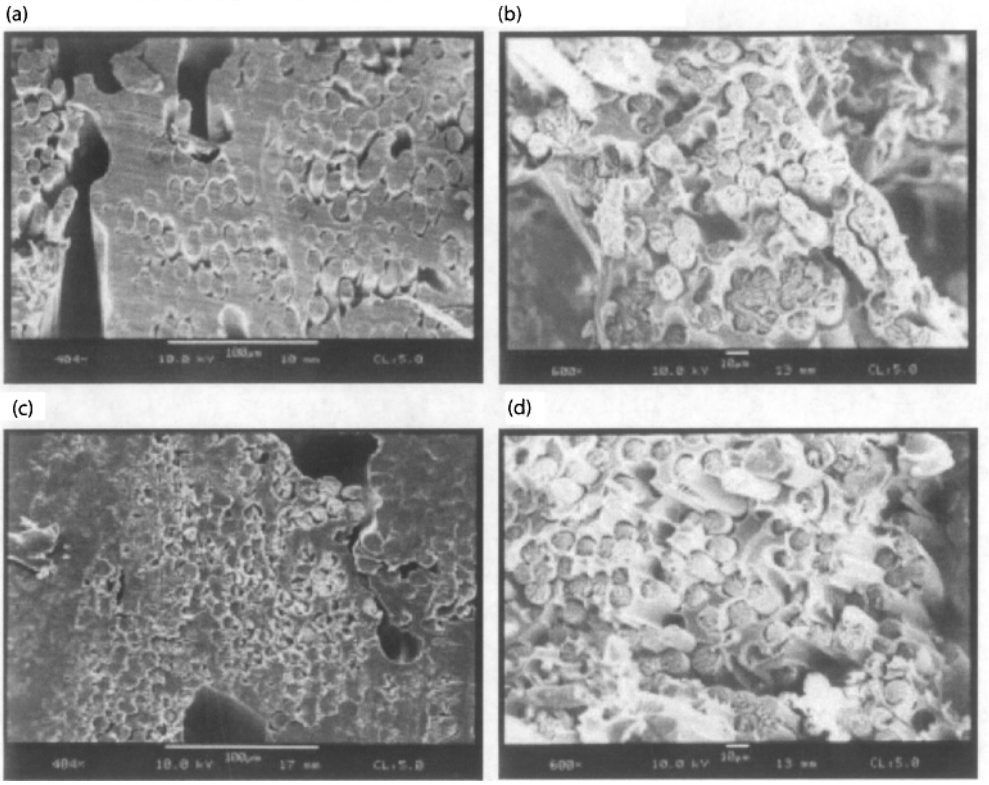


Figure 9.10 (a) SEM micrograph of extrudate cross section untreated composite at 30% fiber loading at a magnification of 100 μm. (b) SEM micrograph of extrudate cross section untreated composite at 30% fiber loading at a magnification of 10 μm. (c) SEM micrograph of extrudate cross section treated composite at 30% fiber loading at a magnification of 100 μm. (d) SEM micrograph of extrudate cross section treated composite at 30% fiber loading at a magnification of 10 μm [74].

of one kind of biofiber with the other being a non-natural/synthetic fiber, for example, natural fiber/glass fiber, talc, and carbon fiber, etc. [80]. These composites can be designed by the combination of a synthetic fiber and biofiber in a matrix and a combination of two biofiber/biofiber in a matrix. Hybridization with glass fiber provides a method to improve the mechanical properties of biofiber composites, and its effect in different modes of stress depends on the design and construction of the composites. Ray *et al.* have reported in detail on the effect of hybridization of glass fiber in thermoset biocomposites [81]. Improvement in the mechanical properties of the low density polyethylene (LDPE) based short banana-glass fiber hybrid composites have been studied by Kalaprasad *et al.* [64–65]. They emphasized that the impediments in biofiber composites were balanced by the combination with the glass fibers and the desired attributes, including optimum performance characteristics of both the fibers, were obtained. Similar results have also been obtained by Bakar *et al.* [82], in the case of oil palm fiber-glass fiber reinforced epoxy resin

hybrid composites, wherein substantial improvement in the tensile properties of the composites have been reported. The enhanced mechanical performance in biofiber-based hybrid composites has also been widely reported by various authors. Extensive investigations of Sreekala *et al.* [55] confirmed that maximum hybrid results are obtained when the fibers are highly strain compatible. Improvement in the impact resistance of banana-glass fiber reinforced PVC hybrid composites has been reported by Yang *et al.* [83].

The effect of hybridization on the water absorption tendency in cellulose fiber reinforced composites has also been studied by many researchers. An attempt to study the moisture uptake characteristics of hybrid systems was performed by Mishra *et al.* [84]. The composite systems chosen were sisal/glass and pineapple/glass fiber reinforced polyester composites. Composites were prepared by varying the concentration of glass fiber and by subjecting the biofibers to different chemical treatments. The authors observed that water uptake of hybrid composites was less than that of unhybridized composites. Similar investigations have also been studied by Sain *et al.* for injection-molded short hemp fiber/glass fiber-reinforced polypropylene hybrid composites [85]. Water diffusion in biofiber-based hybrid composites also depends on the layering pattern in the composites as well as temperature. Various coworkers found that composites with an intimate mixture of glass and banana show the maximum water uptake except at the temperature of 90°C. At 90°C the maximum water uptake was found for composites where there is one layer of banana and another layer of glass. The water uptake follows the same trend as that in all other temperatures up until a time span of 4900 min is reached. The kinetics of diffusion has been reported to be Fickian in nature [85].

9.3.2.1 *Mechanical Properties of Biobased Hybrid Composites of Banana/Glass and PP*

The tensile stress–strain curve of PP and banana fiber/PP (BFPP) and banana/glass/PP (BGPP) with and without MAPP is shown in Figure 9.11. It is observed that, incorporation of banana fibers also induces a brittle fracture in the matrix polymer with a linear deformation at lower strain. However, at higher strain, non-linear deformation characteristics are observed, and continue until there is complete failure in the composite matrix. This non-linear deformation behavior in the composites further confirms micro-crack initiation at the fiber/matrix interface that propagates along the fiber lengths followed by plastic deformation of the matrix polymer, thereby resulting in micro-crack opening and slow crack propagation through the deformed matrix. Finally catastrophic crack propagation takes place through the matrix pulling out the fibers from the matrix. During elastic deformation, the cross sectional area of the specimen decreases uniformly as length increases which is a general characteristic for most ductile polymers. In the cases of the BFPP and BGPP, both with and without MAPP, it can be observed that the increase in load or stress in these composites is faster than that of the pure polypropylene. Furthermore, the effect was more pronounced with the replacement

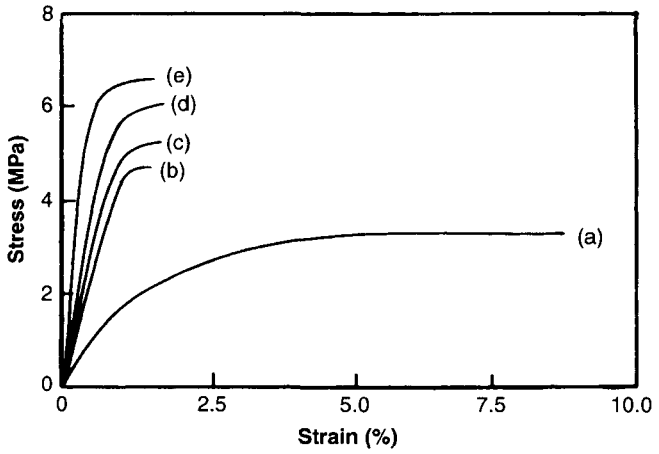


Figure 9.11 Stress-strain of (a) PP (V), (b) PP+30%, (c) PP+15%+15%GF, (d) PP+30% BF+2% MAPP and (e) PP+15%BF+15%GF+2% MAPP.

of banana fiber with glass fiber indicating the influence of hybridization. The failure strain decreased with the addition of the banana fibers indicating immobilization of matrix chains at filler interface leading to premature failure. The characteristic curves of BFPP and BGPP with, and without, MAPP are similar, but the maximum stress of the composite with MAPP is higher than those of the composite without MAPP. The weakness of the composite without MAPP implies poor interfacial bonding between fiber and PP matrix. Therefore, addition of MAPP can improve compatibility and interfacial bonding between the polypropylene and the fibers.

Variation in tensile strength with glass fiber content in BGPP hybrid composites is represented in Table 9.6 [86]. A total fiber content of 30% with MAPP concentration of 2 wt% was optimized and the same has been retained for evaluating the effect of glass fiber addition on the performance of the composites. The mechanical strength of PP increases with the replacement of banana fiber with glass fiber up to 15 wt%. Test results clearly indicate that the tensile strength and modulus of BGPP hybrid composites increases to the tune of 22% and 37% respectively as compared with BFPP composites when both banana and glass fiber are at a ratio of 50:50, i.e., 15 wt% of banana and 15 wt% of glass fiber in the total reinforcement of 30 wt%. The higher tensile strength and modulus of glass fiber mainly accounts for the increase in tensile properties of BGPP hybrid composites over BFPP composite. The addition of glass fiber in BFPP composite also helps to attain a uniform dispersion of banana fiber and avert fiber to fiber contact in the matrix. It is ascribed that the mode of failure of the hybrid composites increases due to the failure of glass fiber which is a low elongated component in the present study. The high modulus and low elongation of glass fiber as compared with the banana fiber, results in an early glass fiber failure at higher load/stress thereby leading to the transformation of a high stress to the weak banana fiber. At low banana fiber loading, the extent

Table 9.6 Mechanical Properties of BFPP Composites and BGPP Hybrid Composites [86].

Sample Type	Banana Content (%)	Glass Content (%)	Tensile Strength (MPa)	Tensile Modulus (MPa)	Impact Strength (J/m)
PP	0	0	32.03±1.06	585.96±2.12	32.45±0.71
BFPP	10	0	37.50±1.1	640±3.2	38.50±0.85
	20	0	41±0.93	815±4.02	42±0.81
	30	0	45.25±0.86	985±4.1	46±1.03
	40	0	39±1.2	1045±3.22	41±1.02
BGPP	25	5	44.50± 0.82	1070±2.23	51±0.96
	20	10	49±0.74	1155±3.53	54.50±1.1
	15	15	57±0.91	1350±5.56	58±1.1
	10	20	53±1.11	1210±4.85	52±0.96
	5	25	51±0.92	1135±3.12	47±0.86
BFPP+1%MAPP	30	0	46.75±1.05	1181±3.01	51±0.99
BFPP+2%MAPP	30	0	50±1.02	1430±2.23	56±0.91
BFPP+3% MAPP	30	0	48±0.93	1300±1.86	50.50±0.96
BGPP+1%MAPP	15	15	59±1.06	1440±2.1	62±1.12
BGPP+2%MAPP	15	15	64±1.2	1692±1.23	69±1.16
BGPP+3%MAPP	15	15	61±0.91	1500±4.3	64±1.08

*Note: ± indicates the standard deviation, the grade of PP used in table 3 and 6 are different hence the properties are also different

of improvement in tensile properties is less compared with 30 wt% of banana fiber loading, which shows us that fiber orientation plays a detrimental role in effecting performance characteristics of the composites. Poor fiber alignment at lower fiber loading results in large free space for fiber movement, thereby reducing the effective stress transfer from the fiber to the matrix. Furthermore, a minimum critical weight ratio of fiber loading is required to reinforce the matrix, hence fiber loading at 10 wt% may not suffice to effectively reinforce the PP matrix and transfer stress [87–88].

It is observed that the banana fiber alone cannot withstand the heavy load and leads to the failure in the composites. In the present investigation, the optimum tensile strength at 15 wt% of each banana and glass fiber loading was observed, which is primarily because at this particular composition banana

fiber can effectively transfer the load from the glass fiber. However, at higher glass fiber loading, the tensile properties decline due to the agglomeration of glass fiber which in turn results in a negative hybrid effect beyond 15 wt% glass fiber loading. An increase in the tensile strength and modulus of BFPP composites, with the addition of MAPP was observed. The tensile strength and modulus of 30 wt% banana fiber reinforced PP at 2 wt% of MAPP loading increased to the tune of 7% and 45.2% respectively as compared with 30 wt% banana fiber reinforced PP without MAPP. In the case of BGPP hybrid composites at 15:15 wt% of banana/glass fiber and 2 wt% of MAPP, the tensile strength and tensile modulus increased to the tune of 12.28% and 25.33% respectively as compared with BGPP without MAPP. The increased performance in the presence of a compatibilizer can also be substantiated with similar phenomenon that the presence of hydroxyl and other polar groups in the banana stem fiber surface leads to weak interfacial adhesion between the hydrophilic fiber and hydrophobic PP matrix which results in de-bonding. In presence of MAPP, the anhydride groups present in the MAPP covalently bonds to the –OH groups of the banana fiber surface and SiO group of glass fiber at the interface and leads to the formation of a stronger interfacial region [86]. Also, maleic anhydride in acid form can suitably interact with the fiber surface through acid-base interactions. The improved interaction and adhesion between the fibers and matrix, either through covalent bonding or acid-base interaction (such as H-bonding), or a combination of both, leads to better matrix to fiber stress transfer. MAPP offers potential covalent bonding between the anhydride group and hydroxyl groups of the banana fiber and SiO groups of glass fiber along with chain entanglement between MAPP and PP chains creating a good stress transfer at the interface.

The notched impact strength also increased with the addition of banana and glass fibers which can be explained by the fact that with the addition of the fibers, the fibers bridge the cracks which increases the resistance to the propagation of the crack. The impact strength of the composites at 30 wt% of banana fiber loading showed an improvement of 19.5% as compared with virgin PP matrix. At high fiber loading of 40 wt%, deterioration in impact strength was observed, which is attributed to a change from a ductile to brittle behavior with increase in fiber content [86]. A similar explanation of the probability of fiber agglomeration in the regions of high stress concentration that requires less energy to propagate a crack could be responsible for failure in the composites.

The SEM micrographs of 30 wt% BGPP hybrid composites of 15:15 wt% ratio of banana and glass fiber (Figure 9.12a) [86] without MAPP indicates poor fiber matrix adhesion. There was evidence of broken fiber ends together with cavities or gaps between fiber and matrix. The presence of these cavities implies that the interfacial bonding between the fiber and the matrix polymer is poor and weak. However, the SEM micrographs of BGPP hybrid composites with MAPP illustrated in Figure 9.12b [86] indicated a reduction in fracture toughness regions of fiber pulled out together with matrix and improved compatibility, which can be explained by the fact that the anhydride group present in MAPP can strongly adhere to the -OH groups in the banana fiber surface fiber

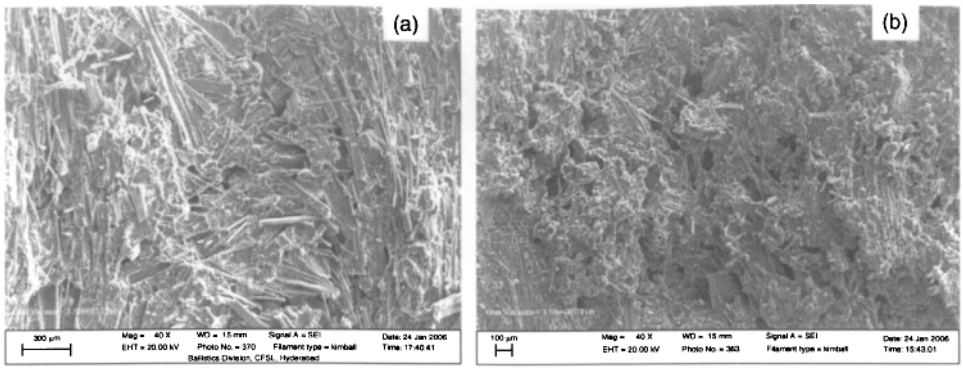


Figure 9.12 SEM Micrograph of (a) BGPP and (b) BGPP+2%MAPP Hybrid Composite [86].

and SiO group of the glass, resulting in improved interfacial adhesion between the PP matrix and both the fibers.

9.3.2.2 *Degradation and Flammability Behavior of Biofiber Composites and Biofiber/Glass Fiber Reinforced Polypropylene Hybrid Composites*

Degradation is a deleterious change in organic chemical structure, physical properties or appearance of a composite, caused by exposure to heat (thermal degradation), light (photodegradation), oxygen (oxidative degradation), micro-organisms (biodegradation), or weathering. Biodeterioration of synthetic polymers can be initiated due to decay of fillers or additives included in plastics. The use of natural fibers as reinforcing materials in polymer matrix composites provides positive environmental benefits with respect to ultimate disposability. Other than flame retardancy, degradation tendency in these fiber reinforced composites is also a deciding factor in the use of these materials for various high performance applications and their ultimate disposal at the end of their life cycle.

Flammability properties of composites play a vital role for the application of these materials in various applications such as automotive components, building materials, and the aerospace industry. As in organic materials, the polymers and the fibers are very sensitive to flame; improvement of flame retardancy of the composite materials has become more and more important in order to comply with the safety requirements of the fiber reinforced composite products. The burning process is comprised of five fundamental steps, which are, heating, decomposition, ignition, combustion, and propagation. Flame retardancy can be achieved by the disruption of the burning process at any of these stages and can lead to the termination of the process before actual ignition occurs [89].

In the present study, the degradability in composites and hybrid composites has also been evaluated under biotic and abiotic environments to evaluate the extent of biodegradation in these materials. Furthermore, the flammability

characteristics of the composites as well as hybrid composites have been studied using horizontal rate of burning and limiting oxygen index tests. Banana fiber PP composites and banana/glass fiber-PP hybrid composites at optimized fiber loading of 30 wt%, fiber ratio of banana/glass of 15:15 and MAPP concentration of 2 wt% exhibited optimum performance, both BFPP composites and BGPP hybrid composites have been used to investigate the degradation behavior and flammability characteristics.

9.3.2.2.1 Determination of Interfacial Bonds in BFPP and BGPP Hybrid Composites Using FTIR Spectroscopy

The existence of interfacial bonds in the BFPP composites and BGPP hybrid composites have been determined using FTIR spectroscopy. The FTIR spectrum of virgin PP, BFPP composites, and BGPP hybrid composites is depicted in Figure 9.13a [90]. Esters have two strong absorption bands arising from C=O and C-O stretching vibrations at shorter wavelengths due to the negative inductive effect of adjacent oxygen atoms that increase the force constant. The

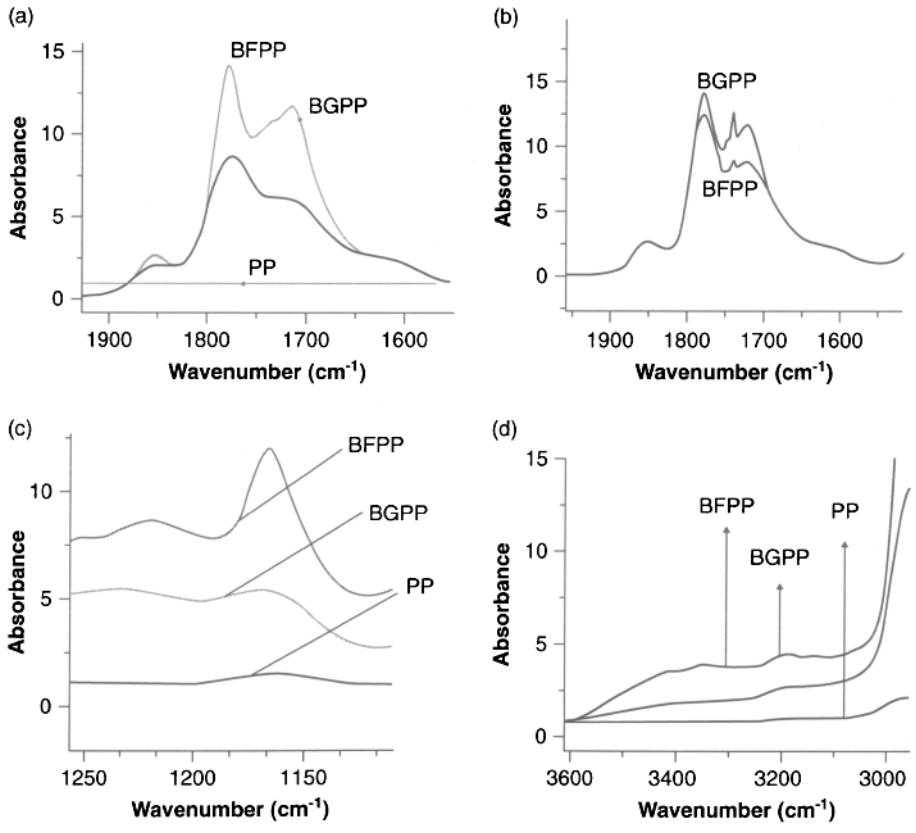


Figure 9.13 (a) FTIR spectra of virgin PP, BFPP composites and BGPP hybrid composites (b) Formation of ester linkage in BFPP composites and BGPP hybrid composites (c) Peaks for free –OH groups in BFPP composites and BGPP hybrid composites (d) Hydroxyl bonding in BFPP composites and BGPP hybrid composites [90].

absence of any observable peaks at 1360 and 1580 cm^{-1} indicates the absence of anionic form of anhydride with one carboxylic acid, i.e., the maleic anhydride is present either in dicarboxylic acid dimer or cyclic form in both BFPP and BGPP samples. This was confirmed by the appearance of peaks at 1780, 1863 cm^{-1} for cyclic anhydride, and 1718 cm^{-1} for carboxylic acid dimmer; this peak was again confirmed by O-H stretching peak (3200–3300 cm^{-1}) because cyclic dimer has a center of symmetry.

Figure 9.13b [90] shows the spectra of both MAPP treated BFPP composites and BGPP hybrid composites where a significantly detectable additional peak from 1740–1750 cm^{-1} was observed. This is possibly due to C=O stretching of ester bond, formed between the coupling agent and fiber in the case of BGPP. However, in the case of BFPP a relatively weak peak could be detected confirming the presence of ester linkage. This behavior is primarily attributed to the presence of glass fiber in BGPP hybrid composites resulting in a strong interfacial balance between the fibers and MAPP [89–90].

Furthermore, it is also assumed that the esterification (chemically joined cellulose moieties) were present in a greater amount in BGPP compared to BFPP; this fact was again supported by the high area under the peak at 1160 cm^{-1} in BFPP for secondary free –OH group, supporting the presence of a higher amount of unreacted cellulose fiber than BGPP as shown in Figure 9.13c [90]. The extent of esterification in BGPP is evident from lower water absorption (0.6%) than BFPP (0.9%) after 24 h at room temperature, which clearly demonstrates the lesser number of -OH groups in BGPP composites due to substitution of banana fiber with less impermeable water resistant glass fiber. The appearance of a less detectable peak at 1050 cm-1 further suggests that primary alcoholic groups of cellulose also participate in esterification and probably the steric hindrance of bulky groups prohibits the reaction. The broad spectrum near 3330 cm^{-1}, present at comparatively lower frequency (bathochromic effect) than the free hydroxyl group (Figure 9.13d) arises from the -OH group in polymeric structure and is due to intermolecular hydrogen bonding by the interaction between -COOH of anhydride and the –OH of fiber.

9.3.2.2.2 Photodegradation

During UV irradiation, cellulosic material undergoes depolymerization or chain scission, leading to the formation of carbonyl groups and fragmentation into non-volatile or volatile products. In the following investigation, the variations in hydroxyl and carbonyl regions as a consequence of photodegradation in BFPP and BGPP, have been monitored using FTIR spectroscopy. The general evolution of the IR spectra of composites upon irradiation in the presence of oxygen is comparable to that of pristine polypropylene and are mainly characterized by an increase of absorbance in the hydroxyl and carbonyl regions (Figures 9.14 a and b) [90]. The photo-oxidation of polypropylene is known to result in the formation of hydroxyl (mainly hydroperoxides and alcohols) and carbonyl groups (mainly ketones, esters, and acids) easily detectable by infrared spectroscopy in the 3200–3600 and 1600–1800 cm^{-1} ranges, respectively. The nature of the main oxidation products can be considered as well-established,

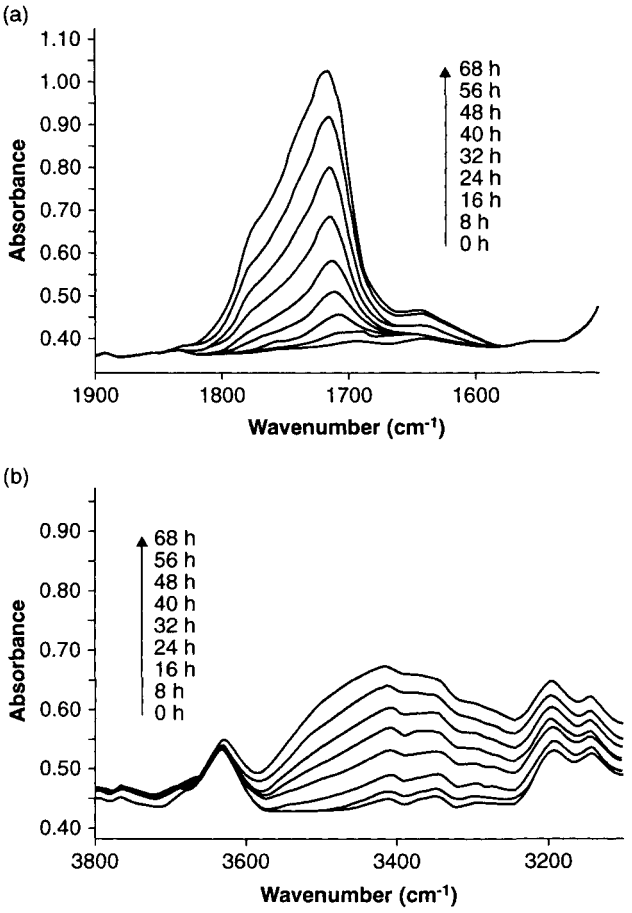
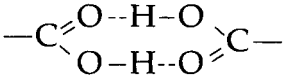


Figure 9.14 Changes in (a) carbonyl group region (b) hydroxyl group region of BFPP composites of PP during irradiation [90].

and the mechanisms by which oxidation occurs is now fairly well understood. In the carbonyl domain the photochemical oxidation of PP leads to the formation of a broad band with several maxima, peaking at 1713, 1735, and 1780 cm^{-1}. The photoproducts corresponding to these absorption maxima have been formerly identified, and it is agreed upon that the main absorption at 1713 cm^{-1} is that of the carbonyl group of carboxylic acids in the dimer form.



(9.4)

The assignment of complex carbonyl absorption peaks in the IR spectra of oxidized samples has been considerably eased by the derivatization techniques. It is noted that MAPP treatment in the BFPP composites has shown that the shoulder on the carbonyl band at 1780 cm^{-1} results from the formation of γ-lactones. The absorption maximum at 1735 cm^{-1} is postulated to result either from the carbonyl groups of esters or from the carbonyl vibration of carboxylic

acids associated to hydroxyl groups in the fiber. In the hydroxyl domain, the broad band peaking up at 3400 cm^{-1} is composed of the O-H absorptions of bonded hydroperoxides and alcohols, with a very weak contribution of the OH absorption of carboxylic acids that have an absorption maximum at lower frequency.

9.3.2.2.3 Rate of Degradation

The rates of photo-oxidation of the various formulations can be compared by measuring the increase of absorbance at 1713 cm^{-1} with the irradiation time. It is important to note that for the formulations containing maleic anhydride (BFRP and BGRP) an absorption band is initially present in the spectrum at the same frequency. This absorption band reveals the presence of carboxylic acid resulting from a partial hydrolysis of maleic anhydride. Because this absorption band overlaps with the bands of the acids coming from the oxidation of polypropylene, all the kinetics of degradation presented here were compared by plotting the absorbance of each sample in the hydroxyl region (at 3400 cm^{-1}). In the case of polypropylene (PP) (Figure 9.15a) [90], one can observe an induction period of 28 h preceding the beginning of oxidation, whereas in case of BFRP an induction period of only 8 h is displayed. This delay is attributed to the disappearance of the antioxidant of processing, which is consumed as a sacrificial additive. Figure 9.15b [90] compares a BGRP and a film of polypropylene with the compatibilizing agent (PP/PPgMA). The results indicate that the induction period is reduced from 20 to 4 h in the presence of both glass and bamboo fibers. The presence of fibers reduces the length of the induction period, suggesting an interaction between the hydroxyl groups of fibers and MAPP. It was also noted that PP composites degrade much more rapidly than pure PP as shown by the absence of an induction period.

9.3.2.2.4 Biodegradation in Compost

The extent of biodegradation in the virgin matrix, BFPP composites, and BGPP hybrid composites, was determined by the percentage weight loss in the samples in periodic intervals up to 6 months in compost. Samples of ~100μm thickness and 5 replicates each, were taken and the percentage weight loss was determined after washing with distilled water and drying in an oven for 24 h at 50°C. The effect of UV irradiation on the biodegradation rate (gravitational weight loss) has also been measured. The degradation tendencies of all the unirradiated and irradiated samples of PP, BFPP composites, and BGPP hybrid composites under composting conditions, are shown in Figures 9.16 a–c [90]. It is evident that the percentage weight loss in the virgin matrix was comparatively less than that of BFPP composites and BGPP hybrid composites, indicating that the fibers degrade initially by providing hydrophilic surface for microbial adhesion. Higher weight loss of BFPP than BGPP after 6 months of composting confirms that the banana fibers are more susceptible to microbial degradation than the glass fibers. Furthermore, in the PP matrix the presence of fibers creates an additional 'facility' for microbial action in the form of degradation products, already generated during its preparation in the presence of free radical peroxides [89–90].

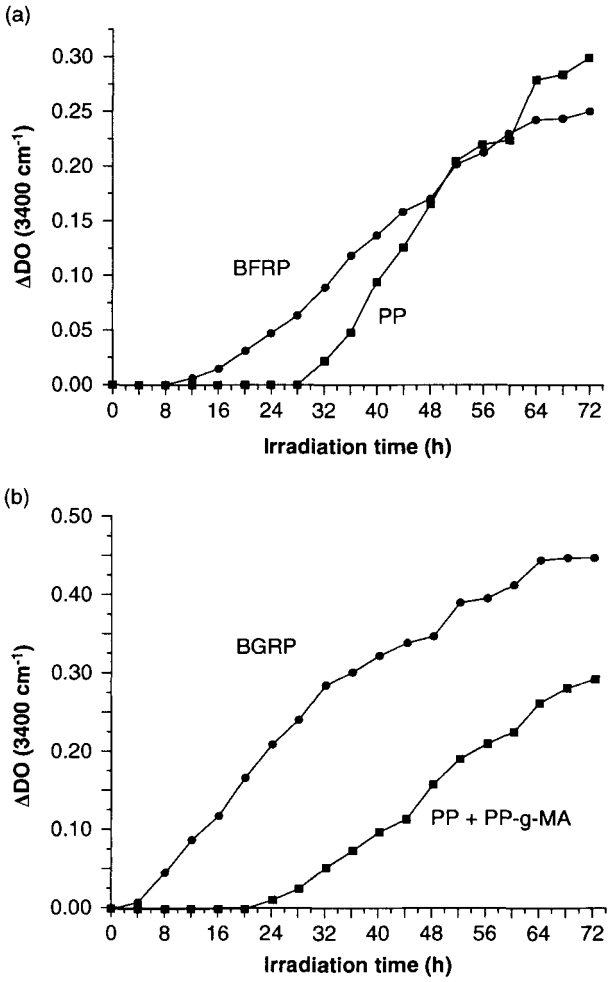


Figure 9.15 Variations of absorbance at 3400 cm^{-1} as a function of irradiation time (a) PP and BFRP (b) PP/PPgMA and BGRP composites [90].

Figure 9.16b illustrates the degradation tendency in the virgin matrix, BFPP, and BGPP after UV irradiation for 20 hours. It shows that during initial exposure for a period of 2–2.5 months, the samples exhibit relatively lesser degradation tendency, which is probably attributed to the formation of initial cross linking via a combination of free radicals. Also, it is believed that at this stage microbes may try to penetrate into the matrix polymer after consuming the nutrients present on the surface, a fact that has been eliminated for longer hour irradiation. Weight loss in the samples substantially increased for 50 and 100 hrs UV treated samples (Figures 9.16 c–d). In all cases the degradation tendency in the filled system was higher than that of the virgin matrix. The samples irradiated for 100 hrs were unrecoverable after 5 months and showed brittleness characteristics during UV treatment. BFPP composites samples could not be recovered after 4 months, whereas the degradation tendency in

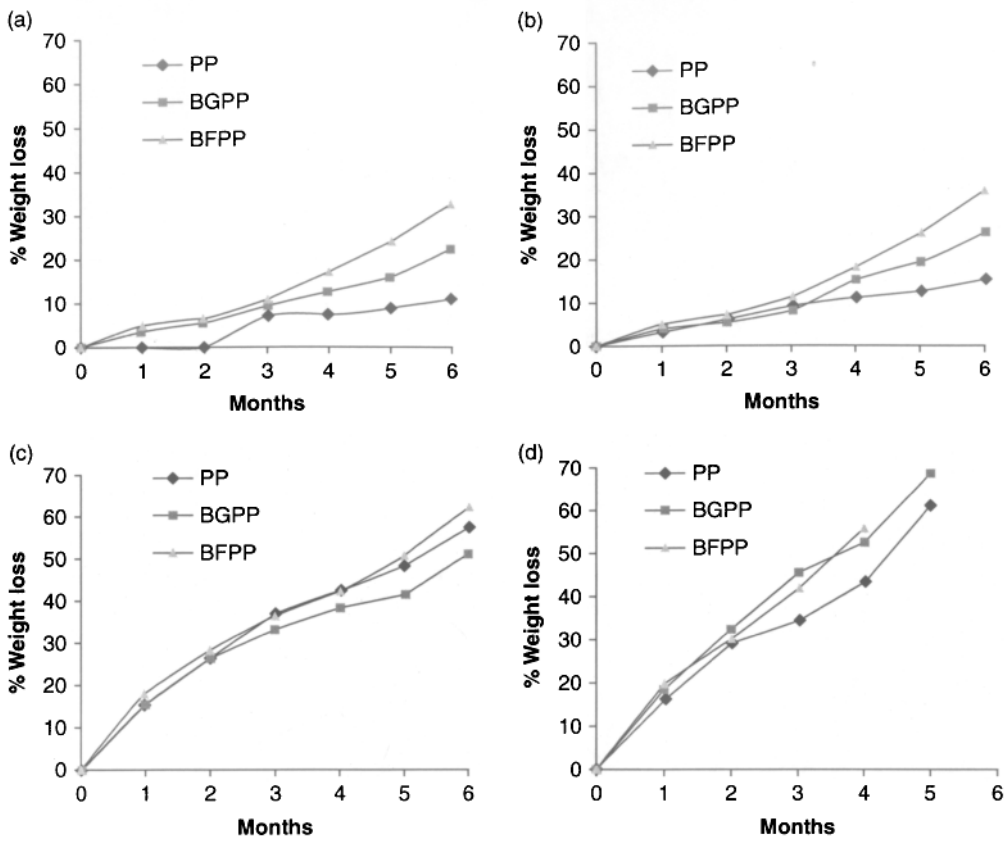


Figure 9.16 Weight loss (a) unirradiated samples (b) hrs irradiated samples (c) 50 hrs irradiated samples (d) 100 hrs irradiated samples [90].

hybrid composites was less, thereby confirming the improved adhesion with the incorporation of glass fiber.

Comparing the percentage weight loss of BGPP vis-à-vis BFPP, it was noticed that weight loss increased around 68% after 5 months in the case of BGPP, and samples could be recovered very carefully, whereas BFPP weight loss ~55%, and could not be recovered in the present system. The protection against brittleness in BGPP must be due to more extensive esterification, that is, more compatibilization; but since ester bonds facilitate biological attack by providing an active site for coenzyme A (Co-ASH) during hydrolysis, these samples underwent high weight loss and stayed in the compost for a longer time, indicating that ester groups play a significant role in the biodegradation. On the other hand, the weight loss of BFPP, comparatively less esterified, was not recoverable, which is probably due to the brittleness characteristics in the samples that primarily resulted via extreme chain scission in polymer matrix. In all cases, photo degradation enhanced degradability in a biotic environment.

9.3.2.2.5 Flammability Behavior of Biobased Composites

The composition of the BSFPP composites and BSGPP hybrid composites used for flammability study is shown in Table 9.7 [90]. The horizontal rate of burning test of the virgin PP, BSFPP composites (Sample type A–G) and BGPP hybrid composites with MAPP is enumerated in Figures 9.17 a and b [90] respectively. It can be seen that BSFPP composites (sample type B) show a higher rate of burning than the virgin polymer, which indicates a higher sensitivity of the banana fiber to flame. However, with the incorporation of 15 wt% of

Table 9.7 Formulation of flame retardant BSFPP and BSGPP hybrid composites [90].

Ingredients	Sample Type						
	A	B	C	D	E	F	G
PP	100	68	53	53	53	68	53
Banana		30	30	30	30	15	15
Glass		–	–	–	–	15	15
MAPP		2	2	2	2	2	2
Magnesium Hydroxide ($Mg(OH)_2$)		–	15	10	10	–	15
Boric Acid (H_3BO_3)		–	–	5	–	–	–
Zinc Borate ($Zn_3(BO_3)_2$)		–	–	–	5	–	–

*NB: A = 100% PP,

B = 68% PP + 30% Banana + 2%MAPP,

C = 53% PP + 30% Banana + 2%MAPP + 15% $Mg(OH)_2$,

D =53% PP + 30% Banana + 2%MAPP + 10% $Mg(OH)_2$ + 5% H_3BO_3,

E = 53% PP + 30% Banana + 2%MAPP + 10% $Mg(OH)_2$ + 5% $Zn_3(BO_3)_2$,

F = 68% PP + 15% Banana + 15% glass + 2%MAPP,

G =53% PP + 15% Banana + 15% glass + 2%MAPP+15%$Mg(OH)_2$

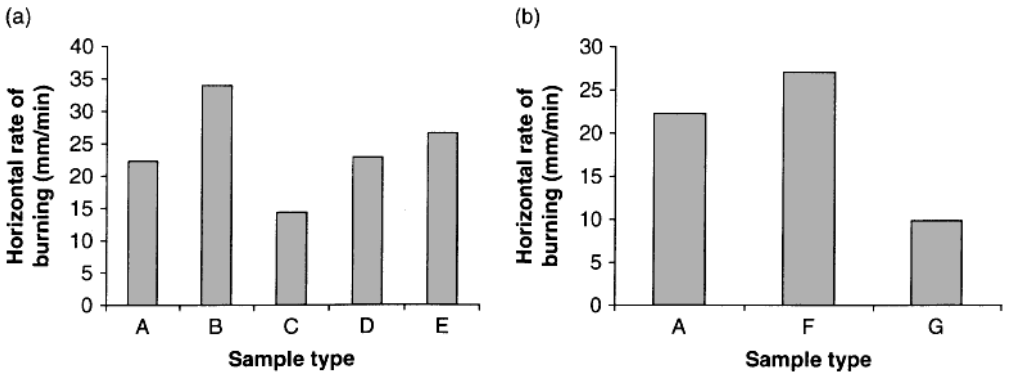


Figure 9.17 Horizontal rate burning of (a) virgin PP and BFPP composites (b) virgin PP and BGPP hybrid composites [90].

magnesium hydroxide (sample type C), the rate of burning of BSFPP is substantially reduced to 50%. This confirms the effectiveness of magnesium hydroxide as a flame retardant additive, by releasing significant amount of water at high temperatures thereby diluting the amount of fuel available to sustain combustion during a fire. Furthermore, it is also assumed that $Mg(OH)_2$ absorbs heat from the combustion zone, reduces the prospect of continuous burning, and produces a char during burning that results in further flame retardant protection and less smoke generation [91].

A combination of magnesium hydroxide with boric acid and zinc borate in the ratio of 10:5 (sample type D and E) exhibits a higher rate of burning compared to the BFPP composite containing magnesium hydroxide (sample type C), but shows a lower rate than that of the composite B. This indicates the retarding effect caused by the combination of the flame retardants instead of the synergism between magnesium hydroxide, boric acid, and zinc borate. Conversely, in the case of BGPP hybrid composites, the rate of burning reduces with the substitution of 15 wt% of banana fiber with 15wt% of glass fiber (Figure 9.17b). This is a further indication that the sensitivity of banana fiber to flame is comparatively higher than synthetic fiber glass. Additionally, incorporation of $Mg(OH)_2$ generates a metal oxide coating that acts as an insulating protective layer during combustion, resulting in a reduced rate of burning.

9.3.2.2.5.1 Limiting Oxygen Index Test Oxygen index tests measure the minimum oxygen concentration required to support combustion; the results of the tests for virgin PP, BFPP composites and BGPP hybrid composites are shown in Figures 9.18 a and b [90]. A sample with high flame retardancy needs a higher oxygen concentration to burn. While composite B requires a relatively low concentration of oxygen to burn, composite C needs the highest concentration of oxygen to burn. Similar to the horizontal burning tests, oxygen index tests also show a reduction in the flame retarding effect rather than a synergetic effect when magnesium hydroxide is used in conjunction with boric acid or zinc borate. In the case of BGRP hybrid composites, a comparatively higher

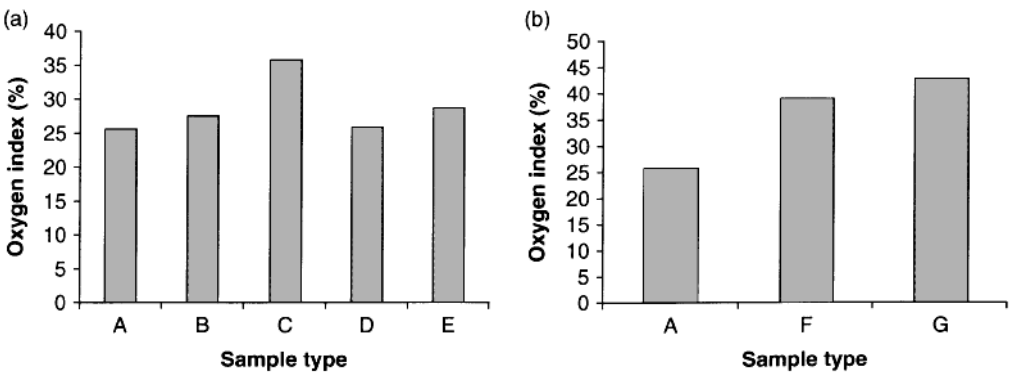


Figure 9.18 Oxygen index of (a) virgin PP and BFPP composites (b) virgin PP and BGPP hybrid composites [90].

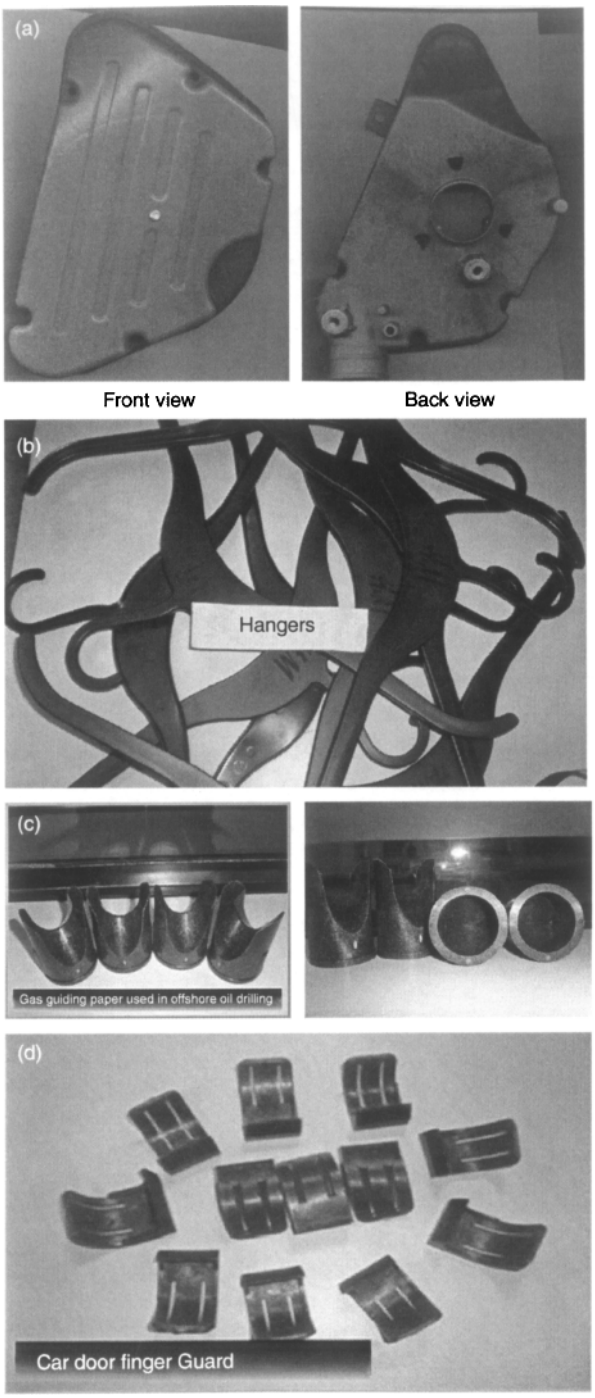


Figure 9.19 Prototypes developed for various end-use applications (a) Motorbike airfilter made up of sisal/PP composites at 30% fiber loading; left side shows the front view while right side shows the back view with metallic inserts (b) Ecofriendly Garment Hangers made out of Sisal/PP composites at 20% fiber loading (c) Gas Guiding paper for offshore oil drilling for application below 100 °C made out of Banana:Glass/PP hybrid composites (d) Automotive Hinge Component made of Banana:Glass/PP hybrid composites.

amount of oxygen is required to support combustion in the samples. $Mg(OH)_2$ incorporated BGRP hybrid composites required the maximum level of oxygen compared to burn, which reveals improved flame retardancy in the system compared to other systems[90].

9.3.3 Prototype Development and Future Recommendations

Because natural fibers are eco-friendly, lightweight, strong, and low-cost, they have successfully replaced glass and mineral fillers in numerous engineering applications, ranging from the aerospace and automotive industries to packaging and construction. Flax, sisal, and hemp are processed into door cladding, seatback linings, and floor panels. Coconut fiber has been used to make seal bottoms, back cushions, and head restraints, while cotton is used to provide sound proofing, and wood fiber is used in seatback cushions. Abaca or banana fiber has also been widely used in underfloor body panels, and manufacturers are implementing natural ingredients into their cars as well. For example, BMW Group UK incorporates a considerable amount of renewable raw materials into its vehicles, including 10,000 tonnes of natural fibers. At General Motors US, a kenaf and flax mixture has gone into package trays and door panel inserts for Saturn L300s and the European-market Opel Vectras, while wood fiber is being used in seatbacks for the Cadillac DeVille and in the cargo area floor of the GMC Envoy and Chevrolet TrailBlazer. Ford mounts Goodyear tires that are made with corn on its fuel-sipping Fiestas in Europe. Goodyear has found that its corn-infused tires have lower rolling resistance than traditional tires, so they provide better fuel economy. The sliding door inserts tor the Ford Freestar are made with wood fiber. Toyota is interested in using kenaf to make Lexus package shelves, and has incorporated it into the body structure of Toyota's i-foot and i-unit concept vehicle. Thus, natural-fiber composites will continue to expand their role in various automotive applications, but only if technical challenges such as moisture stability, fiber polymer interface compatibility, and consistent, repeatable fiber sources are available to supply the automotive manufacturers.

Based on the experimental findings the authors have developed various components in house which can be effectively utilized in various areas (Figure 9.19).

9.4 Conclusion and Future Prospects

The work presented in this chapter shows a great deal of promise regarding the use of biofiber and its hybrid composites. A number of new avenues for future research are also suggested by the studies presented.

1. In studies of the present composite system, the fibers have only been treated with MAPP to improve the interface with the matrix polymer. Various other modification techniques such as isocyanate

treatment, use of silane based coupling agents, and plasma methods can also be employed to modify the interface.
2. The work can also be extended to include the study of other properties such as creep, fatigue, shear strength, chemical resistance and electrical properties.
3. Besides polypropylene matrix, polystyrene which has brittle characteristics can also have a synergistic effect with the incorporation of natural and glass fibers.
4. An extensive survey of literature indicates that there was much less work done in the area of natural fiber reinforced nanocomposites. Hence, the work that is presently done can also be used as a reference in substituting the glass fiber with nanoclays and evaluating the performance characteristics.
5. Attention to these lignocellulosic materials can also be extended to the elaborative study on the biodegradability aspect based on various composting as well as accelerated degradation conditions, in order to cater to existing environmental issues pertaining to the ultimate disposability of plastics based products.
6. Controlled biodegradability after effective use is another important factor in favor of biofiber composite. Life cycle analysis of these products can be carried out to evaluate the durability and consistency of these products for various engineering applications.

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PART 3
MISCELLANEOUS BIOPOLYMERS

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (3–34)

10

Cassia Seed Gums: A Renewable Reservoir for Synthesizing High Performance Materials for Water Remediation

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Abstract

Plants of the *Cassia* genus are widely distributed in tropical and subtropical regions throughout the world and have been extensively investigated chemically and pharmacologically. Besides being a source of secondary metabolites of biological and pharmacological properties, *Cassia* plants are a known source of seed gums which are usually galactomannans having a close structural resemblance to many of the commercial seed gums such as guar and locust bean gums, and are considered non-conventional renewable reservoirs for the galactomannan seed gums. In comparison to other polysaccharides such as starch, the galactomannans are of particular interest because they lead to stereospecific reactions in C_2 and C_3 mannose units and C_4 and C_6 galactose units, and form high performance materials to substitute for expensive petrochemically derived polymeric materials in various fields. Many species of *Cassia* genus have been studied for their seed gum constituents and only a few of them have been chemically modified for commercial applications. There are some recent reports on the use of *Cassia* seed gums as flocculants and adsorbent materials in the removal of textile dyes and heavy metal ions from solutions. These gums work well as flocculant in the removal of dyes both in their native and modified forms. Their efficacy as flocculant can be significantly enhanced by specific vinyl modification. Some of the water insoluble vinyl modified *Cassia* seed gums have also been used as adsorbent in the removal of heavy metal ions from synthetic solutions and real waste water. This chapter will focus on the water remediation by native and chemically modified *Cassia* seed gums in the removal of pollutants from wastewater and synthetic aqueous solutions. Different schematic presentation herein will highlight the various aspects of dye/metal ion removal using native/modified *Cassia* seed gums.

Keywords: *Cassia* seed gums, vinyl modification, water remediation, dyes, metal ions

10.1 Introduction

Recently, in the last five decades or so, the genus *Cassia* has been the centre of attraction for many phytochemists throughout the world and especially in India. It is a large and predominant genus [1, 2] of about 580 species of herbs,

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (269–290)

shrubs, and trees out of which about 20 representatives are found in India. A variety of chemical constituents have been isolated from roots and different aerial parts of the plants belonging to this genus. Some Asian, African, and Indian tribes use *Cassia* species as laxative, purgative, antimicrobial, antipyretic, antiviral, and anti-inflammatory agents. Their seed endosperms contain a substantive amount of the galactomannan polysaccharides. The average degree of galactose substitution varies according to the specific plant species and applications have been evolved to exploit these differences [3]. Many *Cassia* seed gums have been studied and chemically modified for various commercial applications. Seed endosperms of *Cassia abbreviata* [4], *Cassia alata* [5, 6], *Cassia angustifolia* [7–9], *Cassia brewsteri* [10], *Cassia corymbosa* [11], *Cassia fistula* [12–14], *Cassia fistuosa* [15], *Cassia grandis* [16–19], *Cassia javanica* [20–23], *Cassia javahikai* [24], *Cassia laevigata* [25], *Cassia marlandica* [26], *Cassia marginata* [27–29], *Cassia multijuga* [30. 31], *Cassia nodosa* [32, 33], *Cassia ovata* [34], *Cassia obovata* [35], *Cassia occidentalis* [36–38], *Cassia pleurocarpa* [39], *Cassia podocarpa* [40], *Cassia pudibunda* [41], *Cassia renigera* [42], *Cassia reticulata* [43], *Cassia siamea* [44–46], *Cassia sophera* [47], *Cassia spectabilis* [48, 49], *Cassia surattensis* [50], *Cassia saligna* [51], and *Cassia tora* [52–54] have been reported to contain substantial amount of gums having a basic backbone skeleton of $\beta(1\rightarrow4)$ linked mannose units to which α-galactosyl [11] or galactobiosyl residues [7] are glycosidically attached as side chains. Many of these seed gums have been chemically modified and are being used in their native/modified form for various commercial applications.

Like other commercial seed, galatomannans such as Guar and Locust bean gums [9, 55], *Cassia* seed gums have also been found effective in the removal of dyes from the effluents. Due to the presence of metal chelating cis-hydroxyl groups, *Cassia* seed gums are potential candidates to be used as an adsorbent, however, it is difficult to use them under aqueous conditions due to their water solubility. They dissolve on prolonged contact with water and therefore cannot be recycled under aqueous conditions. For designing useful recyclable adsorbents out of these natural resources, several chemical modifications, including vinyl grafting of *Cassia* seed gums have been attempted. On suitable vinyl modification, these seed gums acquire extra functionality for better interaction with the pollutants, besides which their solubility is also suitably tailored for their repetitive use in adsorption-desorption cycles under aqueous conditions. Several reports are available on the modification of these seed gums using various conventional and non-conventional procedures [56–58]. Among the available non-conventional procedures, the use of microwave irradiation is most beneficial as it significantly reduces the reaction time as well as the use of chemicals. In this chapter, basic principles and strategies involved in the preparation of grafted *Cassia* seed gums, their properties, and their use in water remediation will be discussed. For your convenience, the applications of *Cassia* seed gums have been subdivided into two parts; the first part elaborates upon *Cassia* seed gum based flocculants while the second part covers *Cassia* seed gum based metal ion adsorbents.

10.2 *Cassia* Seed Gums Based Flocculants

Flocculation is a process in which finely divided or dispersed particles are aggregated together to form large particles or "flocs" which finely settle and cause clarification of the system. In other words, flocculation makes the suspension non-homogenous on a macroscopic scale [59]. Flocculants are the materials used in fast solid–liquid separations and they act on a molecular level on the surface of the particles to reduce repulsive forces and increase attractive forces. Although water-soluble synthetic polymers find wide application as flocculants, the potential problems associated with their use is their lack of biodegradability. At present, alum is the most widely used coagulant due to its proven performance in treating wastewater of different characteristics and low cost. To partially eliminate the polymerization process that occurs after the alum is added to the water [6], these days polyaluminium chloride (PAC), a polymerised form of alum, is being increasingly used at treatment plants throughout the world. Though the chemical coagulation by alum may be the method of choice for treating wastewater before being fed to the biological treatment units, it also has its drawbacks [61]. The removal process using alum is strongly pH dependent, and the finished water may have high residual aluminium concentration. Moreover, a significant amount of sludge is produced which further complicates handling and disposal procedures. Besides the possibility of Alzheimer's disease due to aluminium, the long-term effects of these chemicals on human health are not known. Furthermore, in many developing countries the cost of importing alum and other required chemicals for conventional treatment might be high and at times prohibitive. To minimize these drawbacks, natural polysaccharides [9, 62] which are extracted from plant or animal life can be workable alternatives to the synthetic polyelectrolytes to work with. They are biodegradable, safe for human health and are endowed with a wider range of effective dosage for the flocculation of various colloidal suspensions. Polysaccharides, owing to their distinctive features such as their ability to undergo different chemical reactions, their biodegradability, and sustainability are attractive, economically viable, and safe substitutes for synthetic flocculants. Many flocculants [63–65] have been prepared by acrylamide grafting onto hydroxyethyl cellulose, amylose, amylopectin, guar gum and alginate. Flocculants have also been designed by the chemical modification of starch [66]. Flocculant cum ion-exchanger from galactomannan guaran has been produced by introducing a sulfonic acid group which, besides flocculating the suspended solids, is capable of removing transition and toxic metal ions [67]. The application of chitosan derivatives as flocculants to treat wastewater have also been studied [68]. It has been established that the poly(acrylamide) modified guar and xanthan gums are effective flocculants, and are being used for the treatment of the industrial effluents containing metallic and non-metallic contaminants [69]. Zhang *et al.* [70] have reported that konjac glucomannan itself is not a good flocculating agent, but after reaction with NaH_2PO_4 behaves as an effective flocculant in water purification.

Owing to the close structural similarity with guaran, *Cassia* seed gums have also been exploited in the removal of pollutants from wastewater. These gums are available in bulk quantities from the seeds of *Cassia* plants and may be engineered with lower amounts by the specific removal of some of their pendant galactose groups using certain R-galactosidases or via chemical modification to introduce new functional groups. Chemical modifications of *Cassia tora, Cassia javahikai, Cassia grandis, Cassia marginata, Cassia siamea, Cassia javanica,* and *Cassia pudibunda* are reported on, and some of these modified products have been tested for their utility in wastewater treatment in comparison to native *Cassia* seed gums under laboratory conditions vis-á-vis synthetic flocculant and adsorbents. Some *Cassia* seed gums have also been used in their native form in the flocculation of dyes (e.g. *Cassia angustifolia*).

The following sections summarize some of the studies where *Cassia* seed gums have been utilized as flocculants for dye removal.

10.2.1 *Cassia angustfolia*

Cassia angustifolia seed gum has been used as a coagulant in wastewater treatment alone and/or in conjunction with PAC. The structure of the polysaccharide from the seeds of *Cassia angustifolia* is well studied [7]. It is a water soluble galctomannan having D-galactose and D-mannose in 3:2 molar ratio and it has an ash content of 0.4%. The main chain of the galactomannan (Figure 10.1) consists of β (1→4)-linked mannopyranosyl units to which (1→6)-linked α-glycosidically bonded galactopyranosyl units or galactopyranosyl units form the branching points.

Native *Cassia angustifolia* (CA) seed gum with 1 mL of PAC stock solution at highly acidic pH (pH = 2.5) has been shown to remove 35% Acid Sendula Red, 22% Direct Kahi Green, and 16% Reactive Remazol Brilliant Violet dyes [9]; while at alkaline pH (pH = 10) under identical conditions, the decolorization efficiency of CA is reported to be about 80%, 99%, and 45% for acid, direct and reactive dyes, respectively. CA acts as a good working substitute

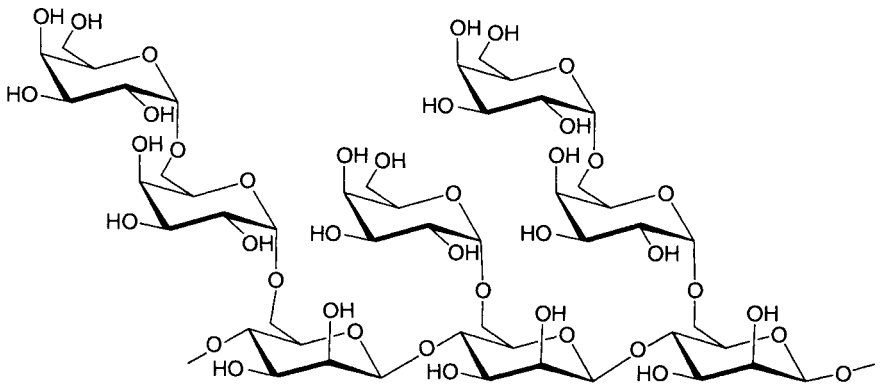


Figure 10.1 Chemical structure of *Cassia angustifolia*.

alone or in conjunction with a very low dose of PAC for the decolorization of acid and direct dyes, but not for reactive dye solutions. It has been shown that the performance of PAC was better with direct dye and that of CA was better with that of acid dye. This application of CA gum in water treatment is a great advantage because PAC treatment generates toxic sludge which is difficult to handle and dispose of.

There is a need for more exploration into such natural resources, which can substitute chemicals in wastewater treatment technologies. Such natural gums may be more effective if extra chelating functional groups are introduced through chemical modification. One such example is the incorporation of the poly(acrylamide) grafts, and such an attempt was made using *Cassia javahikai* seed gum. Details are provided in the following section.

10.2.2 *Cassia javahikai*

Cassia javahikai (CJ) (N.O. leguminosease) is a small tree [71] cultivated in gardens as an ornamental plant. Its seeds contain a substantial amount of galactomannan polysaccharide that has a 1:2 galactose to mannose ratio [72]. Its repeating unit has a linear backbone of $\beta(1\rightarrow4)$ linked mannopyranosyl units with $\alpha(1\rightarrow6)$ linked D-galactose side branches (Fig. 10.2). This type of structure is a fundamental pattern that is found in other commercial seed galactomannans such as guar, carob, locust bean, tara, dhaincha, etc. The structure of the repeating unit of the CJ seed gum is different from that of *Cassia angustifolia* and is shown in Figure 10.2.

Cassia javahikai (CJ) seed gum, both in its native and poly(acrylamide) modified form, has proven to be effective in flocculating different classes of synthetic dyes from wastewater on laboratory scale. Its poly(acrylamide) modification has been done by a conventional thermal grafting procedure using potassium persulphate/ascorbic acid redox initiator in the presence of oxygen as co-catalyst [57]. After the flocculation, the dye concentration in the wastewater sample was monitored at a wavelength corresponding to the maximum absorbance of the dye (λ_{max}) by means of a UV–Vis spectrophotometer. The studied dyes for CJ gum are Direct Bordeaux BW Extra (C.I. Index Red 7) (DBR), Direct Orange (C.I. Index direct orange 26 [29150] (DO), Acid Sandolan Red RSNI [C.I. Acid Red 114] (ASR) and Reactive Procion Brilliant Blue RS (PBB).

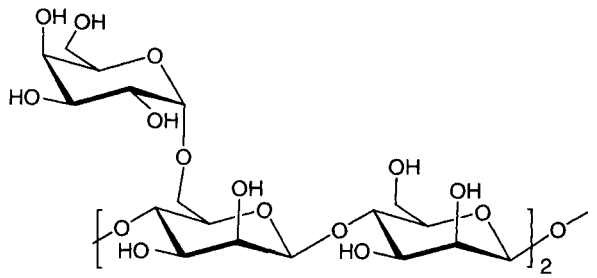


Figure 10.2 Structure of the repeating unit of *Cassia javahikai* seed gum.

Both *Cassia javahikai* seed gum (CJ) and poly(acrylamide) modified CJ seed gum (CJG) behave as good working substitutes for the synthetic coagulants [24] in the decolorization of the studied direct, acid, and reactive dyes. For decolorization of direct dyes (DBR and DO), CJ and CJG alone are sufficient (Figure 10.3), but a small dose of PAC in conjunction with the CJ and CJG is useful for the reactive dyes. The use of PAC with CJ and CJG is helpful in >70% decolorization of all types of dyes (DBR, DO, ASR, and PBB). The variation in pH and dose of CJ and CJG gums (alone or in conjunction with PAC as a coagulant aid) [73] are required to reach optimum results. These parameters also influence the hydrolysis products and hence, the mechanism of coagulation [74]. For polyaluminium chloride (PAC) which is a conventional flocculant, the removal rate increases with the increase in dosage at pH 9.5 (Figure 10.4) and is most effective for DBR followed by DO, PBB, and ASR respectively, at a low

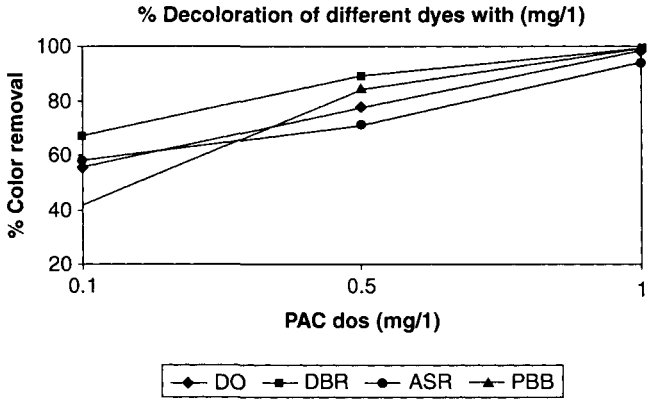
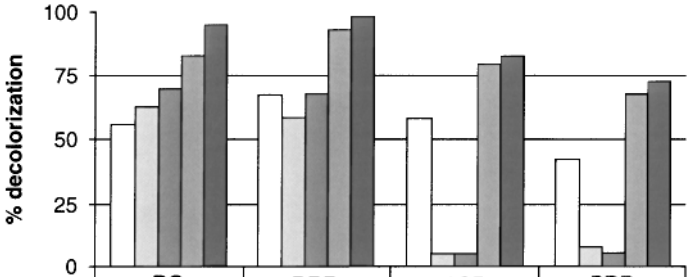


Figure 10.3 Decolorization efficiency of PAC with respect to different dyes.
(Ref. Sanghi et al., Bioresource Technology 97 (2006) 1259–1264)



	DO	DBR	ASR	PBB
0.1 PAC	56	67	58	42
0.5 CJ	63	58	5	8
0.1 CJG	70	68	5	5
0.1 PAC+0.5CJ	83	93	79	68
0.1 PAC+0.5CJG	95	98	82	72

Figure 10.4 % Decolorization of different dyes by CJ and CJG.
(Ref. Sanghi et al., Bioresource Technology 97 (2006) 1259–1264)

PAC dosage of 0.5 mg/L. With the increase in PAC dosage to 1 mg/L almost complete color removal could be achieved for all the dyes except for ASR for which removal was 94%.

Although *Cassia javahikai* and grafted *Cassia javahikai* (0.1–0.5 mg/L) are both very effective in the decolorization of direct dyes (DO and DBR) solution, the grafted gum definitely has an edge over the ungrafted gum. With increase in the coagulant dose up to a certain level (0.5 mg/L), the percent removal of dyes increase followed by a decrease due to the restabilization of the dye solution on the overdosing of the coagulant [75]. Polymer bridging has a big role in the flocculation process, and an excess dose (over optimum amount) of coagulant redisperses the aggregated particle which in turn disturb particle settling [76]. This behavior is due to the repulsive energy between the coagulant and dye in the solution which hinder in floc formation.

pH is also an important criterion [74] for the decolorization studies. Maximum color removal with PAC is reported in alkaline pH range (pH 8.5–9.5) and has been attributed to the adsorption onto hydroxide flocs. After the treatment with PAC, the pH of the dye solution is usually in the range of 9.1–9.3 (for the studied dyes). Because usually pH change does not affect the efficiency of other natural polymers, the coagulation with non-ionic CJ/CJG gums is also not much affected with pH change, and the major mechanism of coagulation is assumed to be polymer bridging. In this mechanism, the charge of the particles and/or polymer does not play any important role.

The contact time is also of much significance in the dye removal. The maximum removal of the PBB, ASR, DO, and DBR dyes was possible after about 1 h contact. Usually the dye removal increases up to an optimum contact time and then becomes constant. Figure 10.5 depicts the dye removal with the change in contact time where three distinct phases are obvious; the first phase (initial steep slope) indicates the interaction of dye molecules with coagulant, which causes destabilization of the particles in suspensions as they began to coagulate. The second phase of the plot shows a slight decrease in percent removal

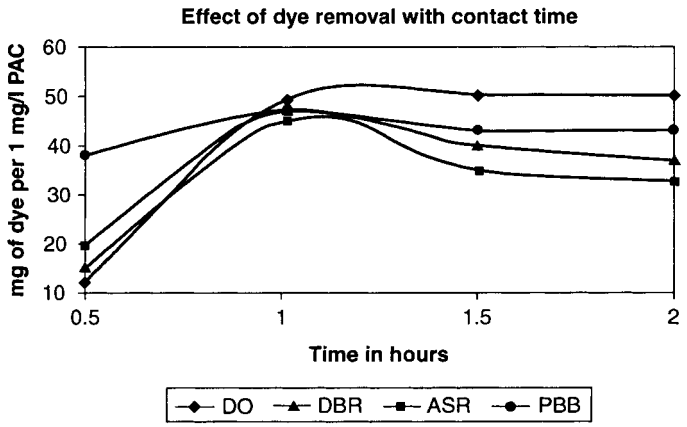


Figure 10.5 Effect of amount of dye removed with time.
(Ref. Sanghi et al., Bioresource Technology 97 (2006) 1259–1264)

of the color due to destabilization of the aggregated particles [76]. The third phase of the plot indicates attained stability by the flocs. The average settling time ranges from 30 min to almost an hour in most of the cases. When CJ alone or in combination with PAC is used, the formed flocs settle faster than that with PAC or CJG alone (Figure 10.5).

10.2.3 *Cassia tora*

Cassia tora gum is derived from the seeds of *Cassia tora* Linn which is a common herbaceous annual weed found growing throughout India. It has a backbone of 1→4 linked D-mannopyranose and D-galactopyranose units [77] where the ratio of D-mannopyranosyl to D-galactopyranosyl units is about 5:1 [78].

Various modified forms of *Cassia tora* seed gum (e.g. carbamoylethyl *Cassia tora* gum (CB-CTG), cyanoethyl *Cassia tora gum* (CE-CTG), carboxymethyl *Cassia tora* gum (CM-CTG), quaternized *Cassia tora* gum (Q-CTG), acrylamide grafted *Cassia tora* gum (CTG-g-AA), and acrylonitrile grafted *Cassia tora* gum (CTG-g-AN) [79–85] have been tested against kaolin under laboratory conditions vis-á-vis poly(acrylamide) based synthetic flocculants.

The flocculation efficiency of these substitution derivatives were very high being 99.65% for Q-CTG, 99.05% for CB-CTG; 98.58% for CE-CTG, and 97.8% for CM-CTG; while for grafted *Cassia tora* the flocculation efficiency were 99.12% for CTG-g-AA and 98.94% for CTG-g-AN. High flocculation efficiencies of these derivatives have been attributed to effective charge neutralization, polymer bridging and large hydrodynamic volume due to the introduction of more hydrophilic groups.

Flocculation efficiencies of most of the chemically modified products of *Cassia tora* are better than Deftech (polyacrylamide based) flocculant, and thus these *Cassia tora* derivatives have the potential to replace synthetic flocculants. These modified products can be exploited for the treatment of many industrial effluents.

10.2.4 Mechanism of Dye Removal by Flocculants

Removal of color from dye solutions is a complex process and involves physiochemical mechanisms of coagulation and/or chelation–complexation type reactions. The dyes, due to the chelation/complex formation reaction with chemical coagulants, lead to the formation of insoluble metal dye complexes which may either precipitate from solution or may be removed by adsorption onto metal hydroxyl species [86].

Seed gum/grafted seed gums are a large molecule with considerably high molecular weight, and have a large number of primary and secondary –OH, sugar ring oxygen, and grafted functionalities which offer hydrogen bonding sites to dye molecules; hence, when they are used in conjunction with PAC for dye flocculation, the removal becomes more effective. The explanation for this result is based on a particle–polymer–particle complex formation in which the polymer serves as a bridge. To be effective in destabilization, a polymer molecule must contain chemical groups which can interact with sites on the surface of the colloidal particle. When a polymer molecule comes into contact with

a colloidal particle, some of these groups adsorb at the particle surface, leaving the remainder of the molecule extending out into the solution. If a second particle with some vacant adsorption sites contacts these extended segments, attachment can occur. A particle–polymer–particle complex is thus formed in which the polymer serves as a bridge.

Since the coagulation dose and coagulation pH influence the type of hydrolysis products, they also influence the mechanism of coagulation which in turn is dependent on dye type and dye structure. Being biodegradable and safe to human health, seed gums and their derivatives can be potential alternatives to conventional chemical coagulants. These natural gums alone and as a coagulant aid can effectively decolorize the dye solutions, and are usable in water treatment to promote a cleaner environment.

It has been established that like other commercial galactomanans, the native and vinyl modified forms of *Cassia* seed gums can be exploited as effective flocculants in the removal of dyes. *Cassia* seed galactomannan have mannan backbone with pendent galactose units which prevent strong intermolecular association of the polysaccharide molecules (due to secondary interactions), and thus, most of the functionalities present in the polysaccharides (native or modified) are exposed to interact with dyes. Comparatively speaking, starch has extensive chain interactions, and therefore in native form has limited potential for hydrogen bonding with dye molecules at the temperature of application [55]. The use of galactomanans derived from renewable plant sources in the dye removal presents a sustainable method of effluent treatment because the *Cassia* plants are widely distributed and are a rich source of seed gums. These bio-derived flocculants are relatively less toxic than the synthetic flocculants and are also biodegradable.

10.3 *Cassia* Seed Gums Based Metal Sorbents

The effective removal of heavy metals from aqueous wastes is one of the most important issues for many industrialized countries. The treatment methods used to remove heavy metals from wastewaters mainly include chemical precipitation, ion exchange, adsorption, or membrane processes [87]. Adsorption using biopolymeric materials offers a technically feasible and economical approach that is also eco-friendly compared to existing high cost technologies. Up until now several biological materials have been investigated for use in the removal of heavy metals including bacteria, yeasts, algae, and fungi [88]. Besides these biomaterials, many seed gums in their modified forms are also used as sorbents for heavy metal ions e.g. guar gum has been used as efficient CR(VI) adsorbent [89-90] after poly(ethylacrylate) and poly(methylacrylate) grafting. Just like guar gum, *Cassia* seed gums also have cis-hydroxyl groups in mannan back bone and are potentially suitable for metal chelation, but due to their solubility under aqueous conditions they require some type of chemical modification before they can be used as adsorbents. Some of the *Cassia* seed gums have been modified and utilized for such applications. The following sections describe these applications of Cassia seed gums.

10.3.1 *Cassia grandis*

Cassia grandis is a small or medium-sized tree found in abundance throughout India. Its seeds contain about 50% endosperm gum and possess all the characteristics of becoming a potential source of seed gum [18]. The purified polysaccharide is a pure galactomannan (mannose: galactose = 3.15), molecular weight (Mw) 80,200 Da, polydispersity (Mw/Mn) 1.35, and intrinsic viscosity [η], 848 mL/g. Like other legume galactomannans it has a $\beta(1\rightarrow4)$-linked main mannan backbone to which galactose units are attached glycosidically at *O*-6 positions. The orthorhombic lattice constants of the hydrated gum are established as: a =9.00, b = 24.81, c =10.30 A°, and the space group symmetry of the unit cell is $P2_12_12$.

Cassia grandis seed gum-graft-polymethylmethacrylate (CG-g-PMMA) samples of different %G have been synthesized using the persulfate/ascorbic acid redox system [19]. For synthesizing maximum percentage grafting and percentage efficiency sample (300 %G and 70.69 %E), 17×10^{-2} M methymethacrylate, 40×10^{-3} M $K_2S_2O_8$, $2.3\ 10^{-2}$ M ascorbic acid and 4g/L seed gum are required in a total volume 25 mL for 1 h grafting reaction time. Being fully water insoluble, this sample has been used as an adsorbent in the removal of lead (II) from synthetic solution as well as from real battery waste. The copolymer efficiency in the removal of the lead increases with the grafting extent and the maximum %G sample has been used for the optimization of the lead sorption by the copolymer. The sorption by the copolymer is pH and concentration dependent, pH 2.0 is the optimum value for the sorption by the copolymer. Adsorption of lead by the grafted seed gum follows a pseudo-second-order kinetics with a rate constant of 4.64×10^{-5} $gmg^{-1}min^{-}$.[1] The adsorption equilibrium data follow the Langmuir isotherm model and based on it, the maximum sorption capacity of the optimum sample is 126.58 $mgg.^{-1}$ Presence of the external electrolyte (NaCl, Na_2SO_4) has a negative effect on the adsorption by CG-g-PMMA as the ions furnished by the electrolytes compete for the sorption sites at the copolymer. Desorption with 2 N HCl elutes 76% of the lead ions from the lead-loaded copolymer (Table 10.1) and it is possible to use the copolymer for at least four cycles, though there is a successive loss in lead sorption capacity with every cycle. The adsorbent has also been evaluated for Pb(II) removal from battery wastewater containing 2166 mg/L Pb(II) (Table 10.2). Optimum Pb(II) binding is observed at highly acidic pH which indicates a significant contribution of nonelectrostatic interactions in the adsorption process. A possible mechanism for the adsorption has been proposed.

Higher %G copolymers show better performance in lead removal which indicates the role of graft chains in the binding. Though infra-red spectrum of lead loaded copolymer indicates some complexation (Figure 10.6) is taking place, a major role in the binding has been attributed to the nonelectrostatic forces. The highest adsorption is observed at acidic pH at which electrostatic attraction between the Pb^{2+} and the protonated copolymer is not possible. It appears that conformation of the copolymer molecules plays an important role in the binding. Seed gums exist as highly disordered random coils [91] in

Table 10.1 Adsorption (AD)–desorption (DS) cycles of the adsorbent. Adsorption condition: Pb(II) 100 ppm, pH 2.0, total volume 20 mL, adsorption dose 100 mg/20 mL, desorption condition: stripping solution 2 N HCl, total volume 20 mL, equilibration time 15 h.

1st Cycle	2nd Cycle	3rd Cycle	4th Cycle
(AD DS)	(AD DS)	(AD DS)	(AD DS)
90.46 76.92	88.12 82.23	87.39 74.7	70.62 64.28

(Ref. Singh et al., J.Colloid Interface Sci., 316 (2007) 224–232)

Table 10.2 Removal of Pb(II) from industrial waste with dilution at pH 2.0, at fixed amount of CG-g-PMMA = 0.05 g, total volume 20 mL, temperature = 30 °C, at 100 rpm in 16 h.

Dilution	Concentration of Pb(II)	Adsorbent Dose (mg)	Adsorption (mg/L)	% A
10 times	216.6	500	130.6	60.29
100 times	21.6	500	15.86	73.22
1000 times	2.16	50	1.894	86.4

(Ref. Singh et al., J.Colloid Interface Sci., 316 (2007) 224–232)

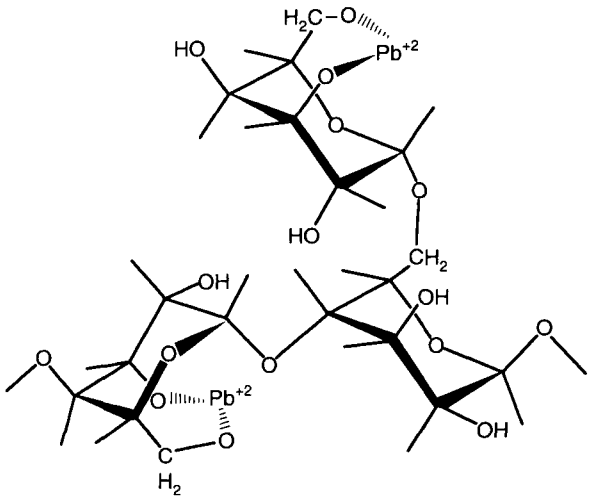


Figure 10.6 Complexation of CH_2OH at biopolymer with lead ions.
(Ref. Singh et al., J.Colloid Interface Sci., 316 (2007) 224–232)

solution where chains in the unsubstituted region can align and pack closely together due to hydrogen bonding and interactive forces. The grafts in copolymer tend to form loops [92] that extend some distance from the surface of the polysaccharide molecule and their ends may dangle. However at acidic

pH, the available hydroxyl groups in the grafted seed gum exist in protonated form and therefore intermolecular hydrogen bonding interaction between the ungrafted portions of the copolymer chains is not feasible. Pb(II) ions get trapped inside the gaps thus created by the disruption of hydrogen bonds (Figure 10.7) and also in between the loops [92]of dangling PMMA grafts. However, a complete understanding of the adsorption by the copolymer at highly acidic pH is left for further investigations.

With real industrial wastewater having a very high concentration of lead, either dilution of the waste or a higher dose of the adsorbent is required for efficient removal. Presence of other ions in the wastewater interferes in the lead adsorption.

10.3.2 *Cassia marginata*

Cassia marginata syn: Cassia roxburghii [93] is a large sized Indian tree having cylindrical and indehiscent long pods with many seeds and a black cathartic pulp which is used as a horse medicine. *Cassia marginata* seed gum-*graft*-poly(methylmethacrylate) (CM-g-PMMA) has been synthesized and evaluated as biosorbent in the removal of Cr(VI) from the synthetic solutions and real wastewater.

In conventional grafting procedures redox initiators are commonly used to initiate the grafting reactions. These initiators produce ions in the reaction medium which may be adsorbed at the copolymer surface to some extent. To avoid this, there has been recent interest in the use of microwave induced grafting reactions. Using microwave irradiation, methylmethacrylate (MMA) has been efficiently grafted onto *Cassia marginata* seed gum in the absence of

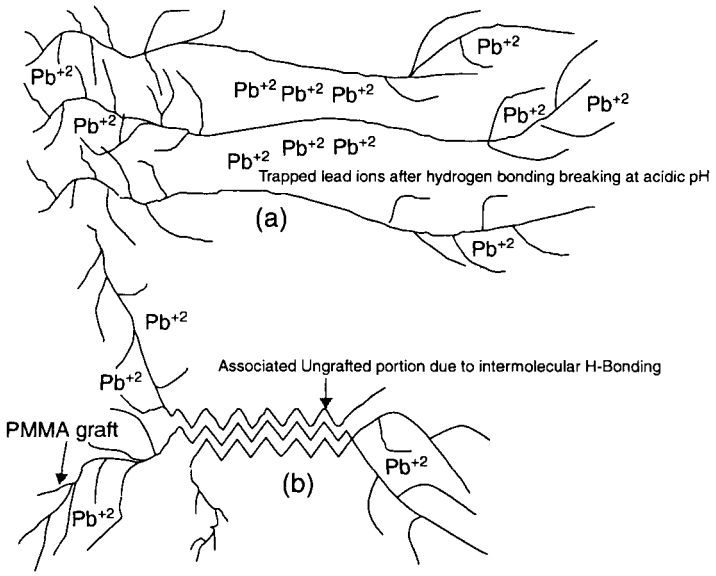


Figure 10.7 Model for interaction between the Pb(II) and graft copolymer: (a) at normal pH, (b) at highly acidic pH.
(Ref. Singh et al., J.Colloid Interface Sci., 316 (2007) 224–232)

any radical initiator in aqueous medium, to obtain *Cassia marginata gum-graft-poly(methylmethacrylate)* (CM-g-PMMA).

Water molecules are present in the reaction mixture as solvent, and being polar they absorb microwave energy and result in dielectric heating which is also contributed by the localized rotation [94] of pendant hydroxyl groups at the seed gum molecule. This results into bond breaking, generating free radical sites responsible for initiating the grafting reaction. Due to the special effect of microwaves [95], a lowering of Gibbs energy of activation for breaking of the bonds has also been reported. In view of these two effects, efficient grafting takes place even in the absence of any catalyst or initiator.

Changing various reaction parameters, graftcopolymer samples of different performances have been obtained. The optimum % grafting (270 %G) and % efficiency (59% E) sample is produced when 4g/L CM gum and 18.0 $\times 10^{-2}$ M methylmethacrylate is exposed for 0.66 min to 40% microwave power.

Water solubility of microwave synthesized copolymer samples decreases with an increase in % grafting, and the sample obtained under optimum grafting conditions shows complete water insolubility and has been used in the Cr(VI) removal from the aqueous solution. The efficacy of the graft copolymer in Cr(VI) removal increases with an increase in the grafting ratio due to the availability of the additional binding sites at the PMMA grafts in the copolymer.

Due to lower % G, the conventionally grafted copolymer sample shows less potential in the Cr(VI) removal as compared to the contemporary microwave synthesized copolymer sample. Moreover, in conventional synthesis of the copolymer, surface adsorption of ions adversely affects sorption ability of the copolymer product. Samples of lower grafting ratio (<100% G) show partial solubility in water and poor ability to remove Cr(VI). The adsorbent copolymer sample before and after Cr(VI) sorption has been characterized using infrared spectroscopy, XRD, TGA, and SEM analysis. Cr(VI) loading at the copolymer has been seen by the presence of Cr-O and Cr=O stretching peaks at 777 and 901 cm^{-1} respectively. Based on the TGA studies, grafted CM gum is shown to be quite thermally stable. After the adsorption of the Cr(VI), structural as well as morphological changes are seen in the copolymer. Structural changes are reflected by the IR spectrum of the Cr(VI) loaded copolymer; however, the SEM picture of the loaded copolymer shows alteration in the surface morphology due to chromium loading.

Maximum Cr(VI) removal has been observed at pH 1.0 and Cr(VI) removal by CM-*g*-PMMA decreases with the variation of pH 1.0–10.0 at 100 mg/L initial Cr(VI) concentration. Total chromium in the equilibrium solution (after the adsorption was detected by the Diphenylcarbazide (DPC) method) indicates that some Cr(VI) converts to Cr(III) during the adsorption and remains in the equilibrium solution along with the remaining Cr(VI). Krishnani *et al.* [96] have made a similar observation for the adsorption of chromium on lignocellulosic substrates. At a low pH, lignin reportedly reduces hexavalent chromium into Cr(III), which is subsequently adsorbed contrary to the adsorption by CM-g-PMMA where the copolymer adsorbed chromium mainly as Cr(VI), while Cr(III), that is generated from the reduction of the Cr(VI) at acidic pH still remains in solution. However, at pH 10 a negligible conversion of Cr (VI) to Cr(III) is reported.

The effect of pH on the removal of Cr(VI) by CM-*g*-PMMA has been interpreted with the help of the surface charge present in the PMMA grafts in an acidic/basic medium. In an acidic medium (97) due to protonation of oxygen lone pairs of PMMA grafts, positive surface charge is developed which increases with a decrease in pH. So, due to the increase in electrostatic attraction between positive surface and negative chromium species ($HCrO^{4-}$/$(Cr_2O_7^{-2})$, adsorption increases with a decrease in pH, Figure 10.8. Cr(VI) which is reduced to Cr(III) at an acidic pH is not adsorbed due to repulsion between the same charges, and remains in the solution.

In acidic medium:

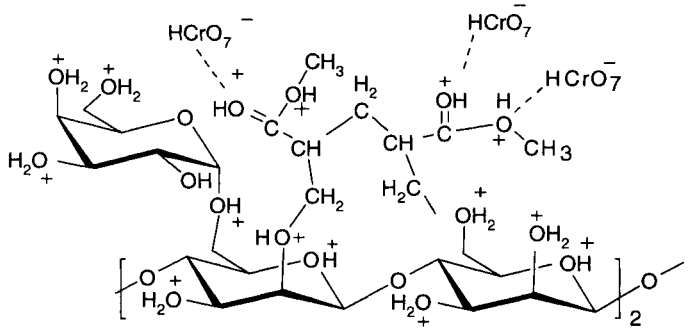


Figure 10.8 Positive surface sites at the copolymer surface responsible for binding.
Singh et al, Ind. Eng. Chem. Res. 47(2007) 5267–5276

But in basic medium [98] base abstracts α-H^+ of the carbonyl group, which makes the surface of CM-*g*-PMMA negative (Figure 10.9). So, due to electrostatic repulsion between the adsorbent surface and negative chromium species, percentage removal of chromium decreases and becomes almost negligible at pH 10.0. The total chromium studies indicates that there is no Cr(VI) to Cr(III) conversion at basic pH.

In basic medium:

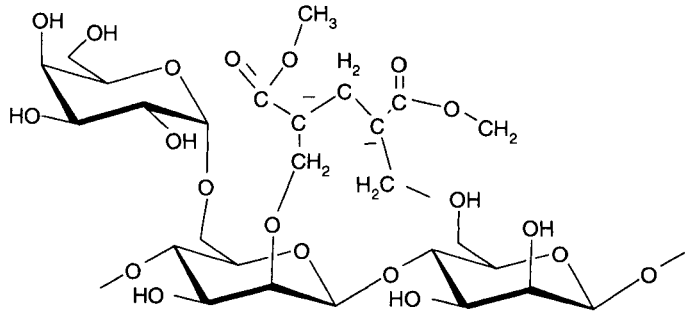


Figure 10.9 Negative surface sites at the copolymer at alkaline pH.
Singh et al, Ind. Eng. Chem. Res. 47(2007) 5267–5276

Adsorption is found dependent on initial Cr(VI) concentration, adsorbent type (%G), adsorbent dose, contact time, and electrolyte concentration.

The overall complex sorption mechanism and heterogeneous nature of the surface sites of the adsorbent are indicated by the isotherm studies and the Q_{max} based on the Langmuir isotherm, is reported to be 185 mg/g. The adsorption follows second order kinetics, the rate constant being 0.10×10^{-5} g/(mg-min) at 100 mg/L Cr(VI) concentration. The adsorbent is also found efficient in Cr(VI) removal from real industrial wastewater. Used copolymer has been recycled after stripping off the adsorbed chromium with 2 M NaOH where after each cycle a successive decrease in the binding capacity has been observed. To understand the advantage of using microwaves in the adsorbent synthesis, the copolymer synthesized using a $K_2S_2O_8$/ascorbic acid redox pair at identical monomer concentration (220% G and 48.6% E) has also been evaluated as Cr(VI) sorbent. Conventionally grafted CM gum is found less efficient in Cr(VI) removal in comparison to that of MW grafted CM gum (Table 10.3).

It is possible to use the copolymer for three consecutive cycles in the removal of Cr(VI), and even in the third cycle, the copolymer removes 54.6% (10.92 mg/g) Cr(VI) after it has been desorbed up to 25.2% (5.04 mg/g). The removal decreases per cycle, suggesting that the copolymer's efficiency continuously decreases up to the third cycle. Both the electrostatic and complexation reactions between the sorbent and the metal ion have been recommended because it is not possible to desorb the copolymer fully. On acid hydrolysis of the used copolymer, the Cr(VI) and PMMA grafts can be easily recovered which is an indication of the economical viability of the copolymer adsorbent.

10.3.3 *Cassia javanica*

Cassia javanica seed gum has been modified by poly(acrylic acid) [99] using conventional persulfate/ascorbic initiated thermal grafting, and by the microwave induced grafting method as in the case of *Cassia marginata*. Once again, with this seed gum at the same monomer concentration, higher %G copolymer sample has been obtained in the microwave method due to the incorporation of higher number/larger PAA grafts. To obtain the optimum %G sample,

Table 10.3 Cr(VI) Removal by Microwave (MW) and conventionally synthesized (CV) graft copolymer using 0.1 g/20 mL adsorbent dose at pH 1.0, temperature 30 °C, rpm 150, and contact time 18 h.

S.No.	Copolymer	Synthetic Solution (ppm)			Real Waste (ppm)		
		10	100	1000	9.09	90.95	909.5
1.	CM-g-PMMA (MW)	1.99	16.94	124.40	1.68	14.17	104.41
2.	CM-g-PMMA (CV)	1.02	5.46	13.06	0.58	2.91	8.91

Singh et al., Ind. Eng. Chem. Res. 47(2007) 5267–5276

a reaction mixture (25 mL) containing 26 × 10^{-2} M AA and 0.1 g CJ gum is exposed to 640 W microwave power for 40 sec. The optimum microwave synthesized copolymer sample shows water insolubility at neutral and alkaline pH, but it dissolves at pH <1 or on overnight stirring at pH 1. The BET surface area of the material is 0.342 m^2/g and cation exchange capacity for potassium, sodium and magnesium ions are 7.6 meq/g, 5.4 meq/g, and 3.8 meq/g respectively. The representative sample (sample having maximum %G) of CJ-g-PAA and mercury loaded copolymer (CJ-g-PAA-Hg) has been characterized using FTIR, XRD, SEM (Fig. 10.10) and BET analyses.

Since the pH_{zpc} of the copolymer is 4.9, at the experimental pH (pH= 6), the copolymer has negative surface sites that are responsible for the adsorption of positive mercury species (Figure 10.11).

The kinetic data for the adsorption of Hg(II) by the copolymer satisfactorily follow both the pseudo-second-order model (where linear plots of t *vs* t/qt) as well as intra-particle diffusion model (up to 3h after that data does not satisfy intra-particle diffusion model, indicating that adsorption in the beginning is diffusion controlled. The correlation coefficients (R^2) and the rate constants for CJ-g-PAA are shown in Table 10.4. While the isotherm studies indicate unilayer

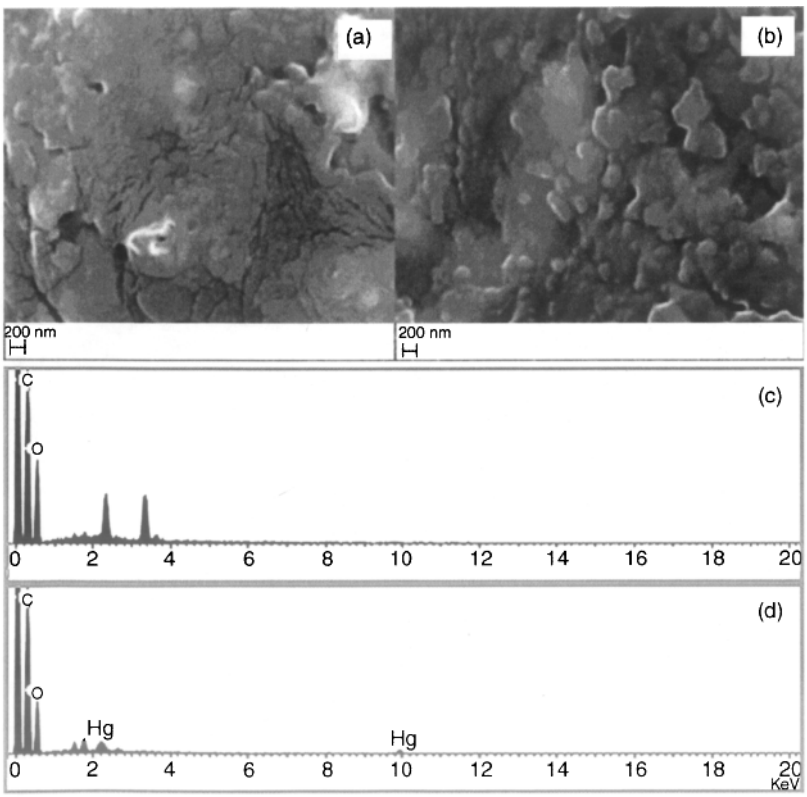


Figure 10.10 SEM picture of CJ-g-PAA (a), Hg(II) loaded CJ-g-PAA (b) and EDX spectra of CJ-g-PAA (c), Hg(II) loaded CJ-g-PAA (d).

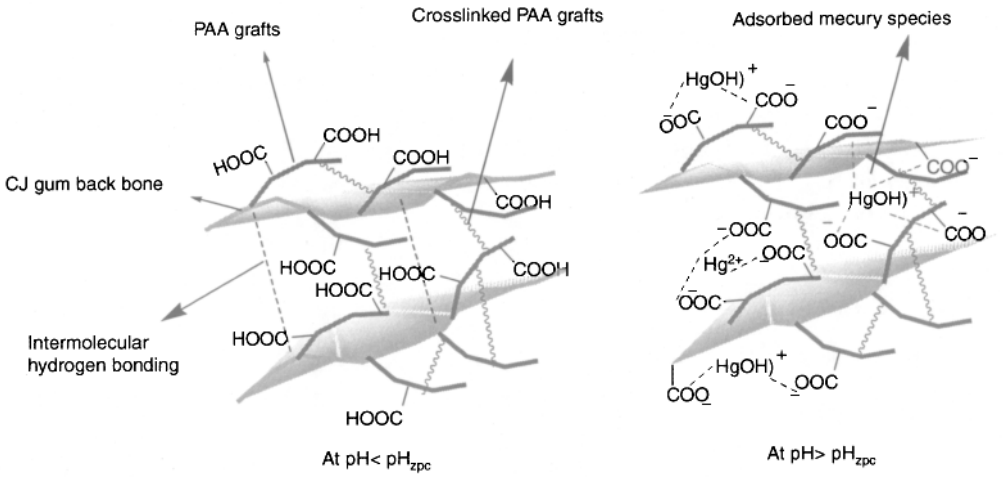


Figure 10.11 Schematic diagram of mercury sorption by the CJ-g-PAA at pH >pHzpc.
Singh et al, Chem. E.J. 160(2010)129-137

Table 10.4 Constants of kinetic models for Hg(II) adsorption on CJ-g-PAA at different Hg(II) concentration, pH 6, temperature 30 °C, rpm 120.

Hg(II) mg/L	Lagergren		Pseudo Second Order			Intra-Particle Diffusion Model	
	R^2	k_L (min^{-1})	R^2	k'(gmg^{-1} min^{-1})	q_e ($mg\ g^{-1}$)	R^2 k_{id}	($mg\ g^{-1}min)^{0.5}$
100	0.9101	4.4×10^{-4}	0.9827	1.97×10^{-4}	50.25	0.9827	2.4277
150	0.9629	2.3×10^{-4}	0.9843	1.36×10^{-4}	74.62	0.9725	3.6712
200	0.9283	2.3×10^{-4}	0.9870	1.36×10^{-4}	95.23	0.9849	4.8632

Singh et al, Chem. E.J. 160(2010)129-137

adsorption by the copolymer because the adsorption equilibrium data better satisfy the Langmuir model and Q_{max} for the sorbent was 135 mg/g.

Hg(II) loaded copolymer sample is easy to desorb with acid and has been recycled for six consecutive cycles. Based on thermodynamic studies, the adsorption is shown to be spontaneous, endothermic, and has good affinity for the metal ion. There is an increased randomness at the solid-solution interface during the fixation of the metal ion on the active site of the adsorbent

10.4 Other Grafted *Cassia* Seed Gums

Many other *Cassia* seed gums have been modified and the properties of the grafted seed gums, such as the viscosity of the gum solutions, and saline and water retention, have been studied to explore the possibility of their exploitation.

10.4.1 *Cassia pudibunda*

This shrub [100] is a rich source for the seed galactomannan and has a close structural similarity with guar seeds which have galactose and mannose in a 1.01:1.50 ratio. It has been grafted with poly(acrylonitrile) using persulphate/ascorbic acid redox initiator [41], and under optimal conditions 294 %G and 85.3% E have been achieved.

10.4.2 *Cassia occidentalis*

Another Cassia seed gum which has been modified is *Cassia occidentalis* (CO) Linn. Kasondi is a common herbaceous annual weed that grows throughout India up to an altitude of 1500 m (101). Every part of the plant is used in different applications in the Indian Ayurvedic system of medicine. The pods are 10–13 cm long and up to 0.8 cm in diameter and contain dark olive green seeds. The *C. occidentalis* seeds are a rich source of galactomannan (w 30% endosperm) [102]. The structure of the backbone of the seed polysaccharide has $\beta(1\rightarrow4)$ linked D-mannopyranose with random distribution of $\alpha(1\rightarrow6)$ linked D-galactopyranose units as side chain (M/G:3.1) (103-105). *C. occidentalis* seed gum has potential use because of its wide availability. The modification of the *C. occidentalis* gum chemically via carbamoylethylation under a variety of conditions has been attempted in order to improve its solubility.

10.4.3 *Cassia siamea*

This seed polysaccharide has been grafted with acrylonitrile under microwave (MW) irradiation without adding any radical initiator or catalyst [46] as needed by other *Cassia* gums. Various properties of the MW synthesized grafted gum such as water/saline retention, water retention after saponification, and viscosity of the gum solutions have been compared with the conventionally synthesized grafted gum and the parent gum.

10.5 Conclusion

This chapter has elaborated how the properties of *Cassia* seed gums in general can be tailored by chemical modification, whereupon they can be exploited as useful dye flocculants and heavy metal adsorbents depending upon their solubility in water.

Even though Galactomannans from *Cassia* seeds are nonionic polysaccharides, their adsorption performance is comparable with that of chitin and chitosan, and superior to other polysaccharides such as corn starch and dextrins. This observation is due to structural hydrogen bonding relationships of the different nonionic polymers. The hydrogen-bonding between the dyes and the galactomannans, and hence their efficiency in effluent treatment, is not effected by variation in pH or by the presence or absence of electrolyte. However, adsorption by vinyl grafted *Cassia* seed gums is pH dependent due

to the incorporation of new functionalities. Some of these *Cassia* seed gums are already being used in industries such as textile printing and food production, and since they have low cost and are readily available they also provide a sustainable method of effluent treatment. There is a possibility that if color can be removed from the dyeing effluent through adsorption, wastewater can be reused several times.

The disposal of the spent adsorbent and recovery of dye/metal ions from it needs to be addressed in more detail. However, the presence of a natural food source with the dye molecules may aid the biodegradation of the dyes in subsequent processes. In any case, the disposal of such adsorbent/dye/metal ion complex would be preferable to the disposal of complexes with alum, silicates, or activated carbon. As such, there is a requirement for these proposed adsorbents to be able to perform at higher concentrations. Furthermore, such higher concentration studies related to adsorption isotherms would provide further insight into the mechanisms and capacity for adsorption of dyes on *Cassia* seed gums.

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11

Bacterial Polymers: Resources, Synthesis and Applications

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Abstract
Polymers derived from biomass such as plants, animals, and microbes have considerable economic and environmental value in addressing global warming and diminishing oil reserves. Hence, for the past few decades extensive research has been done on polymers obtained from plants and animals. Currently the research related to the polymers (e.g. polysaccharides, glycolipids, polyhydroxyalkonates, etc.) obtained from bacteria is rapidly expanding because bacterial species are biodegradable and abundantly available, and easy methods are being developed to grow and harvest them for use in numerous industrial and medical applications. This chapter deals with the conventional methods of extraction, isolation, and biosynthesis, along with the tailor-made synthesis of bacterial polymers and their applications. The future prospective of those polymers will also be discussed.

Keywords: Bacterial polymers, extraction methods, biosynthesis, tailor-made synthesis, applications

11.1 Introduction

Extensive utilization of non-renewable petroleum-based polymers is having a negative impact on fossil while contributing to global warming and other environmental and health problems. To solve these crises and to sustain world development, scientists and technologists have initiated explorations into the alternative use of renewable biopolymers for diverse applications. Biopolymers that originate from plants, animals and microbes are of particular interest, as are polysaccharides (cellulose and starch), protein, oil, chitin, DNA, and RNA. So far, the biopolymers that have originated from plants and animals are being studied for numerous industrial and medical applications. As a result, several products have been developed and commercialized for food

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (291–316)

and non-food applications such as biodegradable plastics, pharmaceuticals, electronics, acoustic devices, bio-fuels, cosmetics, agricultural, and biomedical applications. With an increased demand for renewable polymers, and in order to cope with the decline of plant and animal resources, researchers have focused their studies on abundant bacterial resources to isolate various polymers (Table 11.1). It is well documented that exopolysaccharide, hydroxyalkonates, glycolipids, lipopolysaccharides, and proteins have all been isolated and studied for various applications (Table 11.1).

Table 11.1 List of bacterial polymers and applications.

Polymer Class	Bacteria	Applications
Polysaccharides		
Alginate	Pseudomonas spices and Azotobacter vinelandii	For the production of micro- or nanostructures suitable for medical applications
Cellulose	Aerobacter, Acetobacter, Achromobacter, Agrobacterium, Alcaligenes, Azotobacter, Pseudomonas, Rhizobium and Sarcina	Food (natade coco), Diaphragms of acoustic transducers and wound dressing
Chitosan/Chitin		Used as drug carriers and anti-cholesterolemic agents, blood anticoagulants, anti-tumor products and immunoadjuvants
Curdlan	Agrobacterium biobar and Alcaligenes faecalis	Food additive (for example, as a thickener or a gelling agent)
Dextran	Leuconostoc mesenteroides, Lactobacillusspices and Steptococcus mutans.	This is used in healthy beverages
Gellan	Pseudomonas elodea, Aureomonas elodea and Sphingomonas paucimobilis	This is used primarily as a gelling agent, alternative to agar, in microbiological culture

(Continued)

Table 11.1 (cont.) List of bacterial polymers and applications.

Polymer Class	Bacteria	Applications
Hyaluronic acid	Streptococcal spices and Bacillus subtilis	Play a role in virulence. B
Levan	Alcaligenes viscous and zymomonas mobilis	For use in medical application such as tissue engineering and drug delivery and as well in the food and biotechnology
Xanthan	Xanthomonas campestris	It is used as rheology modifer and food additive
Protein		
Glycoprotein	Aneurinibacillus thermoaerophilus	Used as surfactant
Poly glutamte	Bacillus licheniformis	Aviation fuel storage tanks
Tolin	Francisella tularensis	Exhibit unique immunogenic and biol. activities and may be used in vaccines and in pharmaceutical compns
Peptidoglycon	Borrelia burgdorferi (spirochetes family) and streptococcus faecalis	To study Multi-systemic diseases
Proteinpolysaccharide and Lipopolysaccharides		
Peptidoglycan-polysaccharide	Penicillin treated Bacillus subtilis	Increases the susceptibility of the joint to subsequent injury.
O-specific polymers of lipopolysaccharide	Salmonella typhimurium, Raistonia solanacearum, Helicobacter pylori	Therapeutic applications
Polyesters		
Polyhydroxyalkanoates [PHAs]	Alcaligenes spices A-04, Amphibacillus species	Treating a wastewater that contained xenobiotic orgs.

(Continued)

Table 11.1 (cont.) List of bacterial polymers and applications.

Polymer Class	Bacteria	Applications
PHB (polyhydroxybutyrate)	Pseudomonas pseudomallei	Development of implanted medical devices for dental, craniomaxillofacial, orthopaedic, hernioplastic and skin surgery.
Poly-3-hydroxybutyrate (P3HB)	Azotobacter chroococcum and Pseudomonas putida, Pseudomonas oleovorans, Alcaligenes eutrophus, Zoogloearamigera, Alcaligenes sp. A-04	Medical devices, drug delivery systems
3-hydroxy-n-phenylalkanoic acids	Pseudomonas putida U	Genetic and biochemical studies on a route of the phenylacetyl-CoA catabolon.
Poly-(R)-3-hydroxybutyrate/ polyphosphate (PHB/ polyP) complexes		Removal of metal ions e. g Ca_{2+}, Na_{+}, Sr_{2+}, and Ba_{2+} etc.
Polyhydroxyoctanoate	Pseudomonas oleovorans	Drug delivery systems.
Miscellaneous Polymers		
Stilbene polymer	Gentum parvifolium	NA
Humic polymers	Azotobacter chroococcum	NA [87]
Glycerophosphate polymers	Staphylococcus aureus and Streptococcus faecalis	NA [90]
Polymers of ribitol phosphate	Lactobacillus arabinosus (I), Bacillus subtilis and Staphylococcus aureus	NA [91]
Teichoic acid polymer	Lactobacillus plantarum	NA [113]
Amphipathic polymers	streptococci	Serves as the NVS serotype I antigen.
Polymers of sialic acid	Neisseria meningitides group B and C and Escherichia coli K1 and K92	NA

(Continued)

Table 11.1 (cont.) List of bacterial polymers and applications.

Polymer Class	Bacteria	Applications
1,6-anhydro-muropeptides	Escherichia coli fused with staphylococcal protein A.	NA
O-specific haptenic polymer	Salmonella typhimurium	[114] NA

The goal of this chapter is to provide general information on various bacterial species and the kind of polymers obtained from them. We will also briefly discuss the conventional methods (extraction/isolation), biosynthesis, tailor-made synthesis, and applications of the bacterial polymers.

11.2 Diverse Bacterial Species

Several bacterial species are the resource for various bacterial polymers in addition to organic components such as bacterial phenols [1]. Generally speaking, the kind and the yield of polymer obtained from various bacterial species are different. Until now, various kinds of species were studied to understand the kind of polymers they produced. The different polymers that are produced are polysaccharides, proteins, lipids, and polyesters. The basic structural units of various polymers are presented in Figure 11.1. A wide range of multifunctional high-molecular weight polysaccharides are synthesized by bacteria, which include intracellular polysaccharides, structural polysaccharides, extracellular or exopolysaccharides. These polysaccharides either remain within the bacteria or are secreted into the surrounding environment. The secreted polymers are known as extracellular polysaccharides or exopolysaccharides. The exopolysaccharides mainly constitute glucose, galactose, rhamnose, fructose, and mannose as neutral sugars along with glucoronic and galacturonic acids and a diacid hexose. Several polysaccharides are produced by a spectrum of bacterial species. The structure of the polysaccharides varies due to its surroundings, as well as on the basis of the feed ratio of carbon, oxygen, nitrogen, amino acids, glucose, galactose, etc. [2, 3].

11.2.1 Polysaccharides

The kind of polysaccharides that are isolated from different bacteria are as follows: *Alginate*, a linear copolymer with (1-4)-linked β-D-mannuronate and its α-L-guluronate residues that is produced by two bacterial genera Pseudomonas species and Azotobacter vinelandii [4]. Bacterial alginates are useful for the production of micro- or nanostructures suitable for medical applications. *Cellulose*, a β (1→4) linked D-glucose unit obtained from Acetobacter xylinum. Cellulose of plant origin is usually impure as it contains

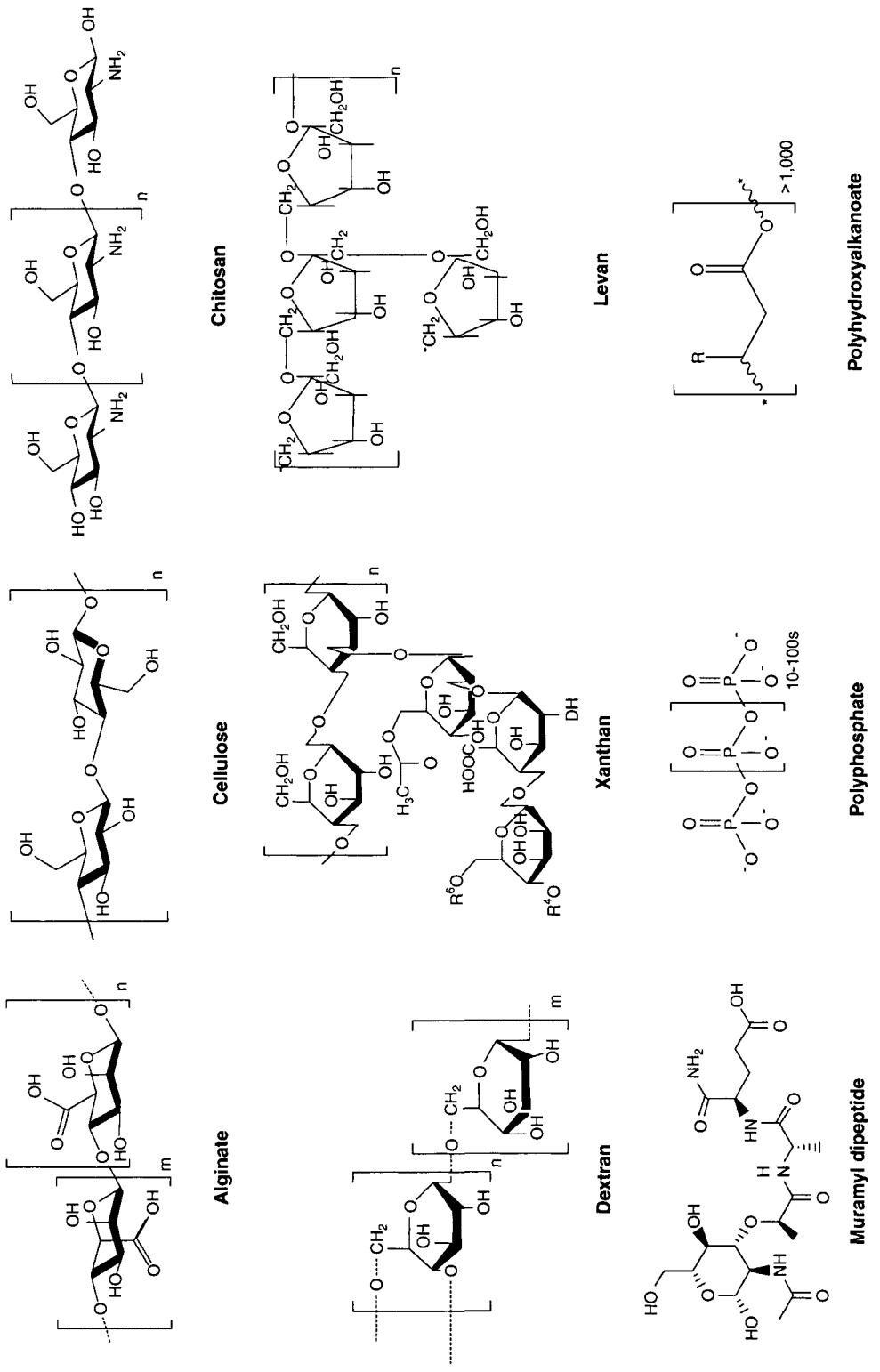

Figure 11.1 Structures of bacterial polymers.

hemicelluloses liginin, pectin, and other substances, while microbial cellulose is quite pure with long chains and containing more water. Bacterial cellulose is isolated from the genera Aerobacter, Acetobacter, Achromobacter, Agrobacterium, Alcaligenes, Azotobacter, Pseudomonas, Rhizobium, and Sarcina synthesize cellulose. Cellulose for commercial purpose is produced by Acetobacter xylinus and Acetobacter xylinum. The bacterial cellulose is more advantageous than plant cellulose due to its good properties [4]. However, it is not cost effective due to high price, low yields, and lack of large scale production methods. The fibrous form of bacterial cellulose is represented in Figure 11.2. *Chitosan* is randomly distributed with deacetylated and acetylated units of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine respectively. It is isolated from the Mucorales species [4]. *Curdlan* is a high molecular glucose polymer (β-(1, 3)-glucan) with β-(1, 3)-linked glucose residues. Curdlan gels on heating and these gels are elastic. Bacteria such as Agrobacterium biobar and Alcaligenes faecalis produce curdlan [4]. *Dextran* is branched glucan (glucose polymer) with a molecular weight ranging from 10–150 kilodaltons. The straight chain consists of α-1, 6 glycosidic linkages between glucose molecules, while branches begin from α-1, 3 linkages. It is produced by bacteria Leuconostoc mesenteroides, Lactobacillus species, and Steptococcus mutans. It is used in healthy beverages [4]. *Gellan*, also known as gellan gum, is produced by bacteria such as Pseudomonas elodea, Aureomonas elodea and Sphingomonas paucimobilis [4, 5]. The repeating unit of the polymer is a tetrasaccharide [D-Glc(β1→4)D-GlcA(β1→4)D-Glc(β1→4) L-Rha(α1→3)]$_n$ which consists of two residues of D-glucose and one of each residues of L-rhamnose and D-glucuronic acid. Currently gellan gum is marketed by a few suppliers such as Applied Gel, Phytagel, or Gelrite. It is used primarily as a gelling agent, and an alternative to agar, in microbiological culture. *N-acetyl heparosan* is obtained from Escherichia coli and Pasteurella muitocida. It is unsulfated and similar to hyaluronic acid and heparin. The heparosan polymer has greater stability within the body as it is not the natural final form of the sugar, and the body has no degrading enzymes or binding proteins that lead to loss of functionality [4, 6, 7].

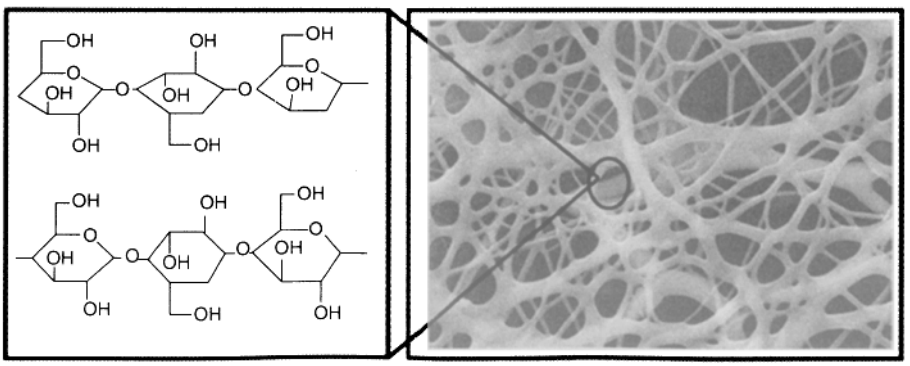

Figure 11.2 Bacterial cellulose fibers.

Hyaluronic acid is an anionic, nonsulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues. It is obtained from Streptococcal species and Bacillus subtilis and plays a role in virulence. B. subtilis produces human grade hyaluron [4]. *Kefiran* is a pale yellow polysaccharide which can gel. It is produced by Lactobacillus kefiranofaciens and Lactobacillus delbrueckii [4]. *Levan* is a biopolymer of homopolysaccharide with D-fructofuranosyl residues joined by 2, 6 with multiple branches by 2, 1 linkages. Microorganisms such as Alcaligenes viscous and zymomonas mobilis produce levan by genetic engineering [4] for use in medical applications such as tissue engineering and drug delivery, as well as in the food and biotechnology industries. *Xanthan* is a gum. It is produced by fermentation of glucose or sucrose by bacteria, Xanthomonas campestris. It is used as a rheology modifier and food additive [4] Exopolysaccharides are also isolated from marine bacterial species (Alteromonas macleodii, sub species fijiensis) [8], and bacteria of the human intestine (Bifidobacterium longum subsp. Longum IPLA E44) [9]. Polysaccharides with surfactant properties were produced from Microbacterium species [10], acidic polysaccharides containing neutral sugars (major) and uronic acid (minor), and capsular polysaccharides are isolated from Klebsiella species [11, 12]. *O-polysaccharide* is obtained from Cronobacter muytjensii strain 3270 [13[a]]. This bacterium is isolated from powder infant formula from Denmark. This polymer is a linear unbranched polymer of a repeating pentasaccharide unit composed of 2-acetamido-2-deoxy-D-galactose (D-GalNAc), 2-acetamido-2-deoxy-D-glucose (D-GlcNAc), 3-acetamido-3-deoxy-D-quinovose (D-Qui3NAc), L-rhamnose (L-Rha), and D-glucuronic acid (D-GlcA) in equimolar ratio. A polysaccharide of D-galactosamine polymer with compound ratio of galactosamine-acetyl-phosphorus (1.0:1.0:0.35) is isolated from the cell wall of Neisseria sicca [13[b]]. *Extracellular glycol* polymers are isolated from Ralstonia solanacearum [14]. Escherichia coli promote the synthesis of a polysaccharide adhesion, which is composed of unbranched β -1, 6-N-acetyl-D-glucosamine, biofilm formation [15]. Polysaccharide [β-1, 6-linked glucosamine substituted with N-acetyl and esterified O-succinate] is used for adhesion of bacteria produced by Staphylococcus epidermidis [16]. Novel polysaccharide antigen termed *as poly N-succinyl β-1, 6 glucosamine* (PNSG) is produced from Staphylococcus aureus [17]. A polysaccharide polymer of β -1-6 linked N-acetyl glucosamine residues (PNAG) is also obtained from Staphylococcus aureus [18]. *Acidic heteropolysaccharide* composed of neutral sugars (29.4%), uronic acids (14.2%), and amino sugars (0.93%) is isolated from Enterobacter cloacae WD7, Enterobacter agglomerans WD50, and Pseudomonas alcaligenes WD22, E. cloacae WD7 [19]. Exocellular glucomannuronic type polymer is isolated from bacterial Xanthomonas fuscans XF 1 microorganism [20]. Polysaccharide containing O-acetylated glycero-galacto-heptose as the main constituent and an unknown aldose is isolated from Eubacterium saburreum [21]. *Polyglucan* polymers were produced by 4 strains of Streptococcus mutans [22]. *Polyglucose* is obtained from Streptococcus salivarius of human saliva [23]. *Galactan* is produced by the integral part of Mycobactrial cell wall [24]. *Polymannan O-antigenic polysaccharides* (O-PSs) are obtained from Escherichia coli O8 and O9a [25].

11.2.2 Proteins

Proteins are lengthy chains of amino acids, which are synthesized in all living beings and are essential for growth and development. Plants and animals are an abundant source for proteins as compared to bacterial proteins. Bacteria synthesize both toxic and non-toxic proteins. Researchers think it is most imperative to understand bacterial proteins because bacteria play a very active role in human health. By acquiring information regarding these proteins more data can be extrapolated about the proteins associated with larger organisms, including humans.

Bacterial protein has the ability to bind with other proteins involving very strong links between two different proteins. As a result of binding, the proteins trigger a reaction altering the immune system response to an infection leading to a disease. Over time, many bacteria have evolved to produce proteins, which target particular locations on human and animal cells. Further investigation on the structural properties of bacterial proteins would enable researchers to understand their role in the development of disease, and accordingly they can develop medications such as antibacterial drugs to target specific organism.

This chapter details only non-toxic proteins isolated from the cell walls of various bacterial species, as the studies dealing with toxic bacterial proteins is vast and requires a separate chapter to understand their effects on living organisms.

From a survey of existing literature it can be seen that the yields of non-toxic proteins and protein polysaccharides are low when compared to polysaccharides or esters. Hence, the studies on bacterial proteins are limited. The various proteins and protein polysaccharides obtained from bacteria are as follows: *Glycoprotein* polymer (MC3B-10) is obtained from Microbacterium species; this polymer, along with polysaccharide, produces high surfactant properties [10]. The glycoprotein covered on the surface of Aneurinibacillus thermoaerophilus DSM 10155 is isolated. This is the only completely water-sol S-layer glycoprotein to be reported to date [26]. The high molecular weight of *poly glutamate* polymer is obtained from Bacillus licheniformis [27]. *Tolin* is one of the glycoprotein obtained from Francisella tularensis [28]. *Peptidoglycon*, also known as murein, consists of sugars and amino acids which are obtained from Borrelia burgdorferi (spirochetes family) and streptococcus faecalis [29-31]. *Muramyl dipeptide* is obtained from streptocci species [32]. Poly-β -D-glutamic acid and poly-β -L-glutamine are produced by the genera Xanthobacter, Flexithrix, Sporosarcina, and Planococcus [33].

11.2.3 Protein-polysaccharide and Lipopolysaccharides

Peptidoglycan-polysaccharide (PG-PS) fragments are isolated from S. pyogenes [34] to study the reactivation of induced inflammation. *Peptidoglycan* polymers [35] are isolated from bacterial cell walls. The peptidoglycan and related polymers are obtained from penicillin treated Bacillus subtilis [36], Streptococcus pyogenes, Staphylcoccus epidermis, Nocardia corynebacteriodes and Streptomyces

gardneri [37]. Capsular polysaccharides and exopolysaccharides are comprised of proteins and glycoproteins, which are extracted from Bacillus firmus and Bacillus sphaericus [38]. Peptidoglycan-polysaccharide and its complexes are obtained from the cell wall of Streptocci (present in humans) and Streptococcus pyogenes, Type 14 [39, 40, 41[a]]. Peptidoglycan and polysaccharide antigens are obtained by the biosynthesis of cell walls of protoplasts of type III group Bacillus streptococcus [30].

O-specific polymers of lipopolysaccharide are isolated and characterized from Pseudomonas cepacia [42]. Lipopolysaccharide is produced from Salmonella typhimurium, Raistonia solanacearum, Helicobacter pylori [32, 14, 43, 44]. Lipopolysaccharides are synthesized biologically by Salmonella typhimurium [45].

11.2.4 Polyesters

11.2.4.1 Polyhydroxyalkanoates (PHAs)

PHAs are linear polyesters that are produced naturally by bacterial species due to an inadequate supplement of nitrogen [46, 47]. The PHAs are biodegradable thermoplastics; if T_g, T_m, and the degradation rate is controlled, they can replace polyethylene. Among the various PHAs, poly (hydroxybutyrate) (PHB), poly (hydroxybutyrate-co-hydroxyvalerate) (PHBV), poly (hydroxybutyrate-co-hydroxyhexanoate) (PHBHx), and polyhydroxyoctonate (PHO) can be produced in sufficient quantity for utility applications. PHB and PHBV are used most extensively for biomaterial investigations. Several bacterial species are involved in the production of various PHAs and its copolymers. The various PHAs and their bacterial origin are detailed below:

Alcaligenes species in the activated sludge microbial consortium are identified as the main genus accumulated with these polyhydroxyalkanonates [11, 48]. PHB is isolated from Alcaligenes species A-04, and Amphibacillus species (found in soil) [48, 49] in the studies that involved characterization. It is well reported that with a variation of carbon, nitrogen, and sucrose content, the yield of PHB varied [50]. A new bacterial strain (Genus Acidiphilium), designated as strain DX1-1, was isolated from acid mine drainage from the Dexing mine in the Jiangxi Province of China. There is a published article which details the extraction of PHB and explains growing conditions of these bacteria [51]. A simple method for the isolation of PHA from the bacteria Sinorhizobium meliloti (MTCC 100) is explained in the article [52]. PHB is also isolated from Pseudomonas pseudomallei using the synthetic enrichment media [53].

The *Poly-3-hydroxybutyrate* (P3HB) form of PHB [54, 55, 4, 56, 57] is probably the most common type of polyhydroxyalkanoate which is isolated from Azotobacter chroococcum, Pseudomonas putida, Pseudomonas oleovorans, Alcaligenes eutrophus, Zoogloea ramigera, and Alcaligenes sp. A-04 [58, 59, 55, 60, 61, 62, 56].

PHB-co-PHV [54] is obtained from Azotobacter chroococcum [58, 63]. The biodegradation is slower for the copolymers than poly-3-hydroxybutyrate. 3-hydroxy-n-phenylalkanoic acids and 3-hydroxyaliphatic acids are obtained from Pseudomonas putida [59]. Poly (3-hydroxyoctanoic acid) and poly (6-hydroxyhexanoic acid) and poly (3-hydroxyoctanoic acid) [64], Poly-(R)-3-hydroxybutyrate/polyphosphate (PHB/polyP) complexes are isolated from the plasma membranes of bacteria [65, 66]. Polyhydroxyoctanoate is produced by feeding octanoic acid to Pseudomonas oleovorans [67].

In addition to the above mentioned polymers, there are also other polymers obtained from bacteria. However, the studies on these polymers are very limited. Following is a list of these polymers and their bacterial species for the information of the readers:

Viscous unidentified polymer is produced by anaerobic bacterial isolate, designated strain JEL-1. The polymer production is controlled by changing the content in the liquid medium with amino acids, glucose, and nitrogen [2, 33]. Unspecified polymer is produced by thermo tolerant bacteria Bacillus subtilis WD90, B. subtilis SM 29, and Enterobacter agglomerans SM 38 [68], which are used as flocculants. Stilbene polymer (Gn-3) is isolated from Gentum parvifolium. Gn-3 was found to inhibit the development of liver injury caused by carbon tetra chloride, N-acetyl-p-aminophenol (APAP), and Bacillus Calmette-Guerin (BCG), and bacterial lipopolysaccharide (LPS) in a study done on mice. This means that Gn-3 has liver protective effects [44]. Adhesive polymer was obtained from Pseudomonas species. NCIMB2021. The author of this published report compared the difference in adhesive property of this polymer with the adhesive polymer of the biofilm matrix [69]. Friction-reducing polymer designated as PS-6 is obtained from fish skin bacteria. There were studies done dealing with isolation and characterization [70]. *Humic* polymers, which consist of mono and dihydroxyphenolic acids, are obtained from Azotobacter chroococcum grown on BzONa. The research is done into degradation studies [71]. A new bacterium is isolated from estuary produced sugar polymers. The method of isolation of these polymers is explained in a report [41b]. Staphylococcus aureus and Streptococcus faecalis produced *glycerophosphate* polymers [72]. Polymers of *ribitol phosphate* [teichoic acid] are isolated from Lactobacillus arabinosus (I), Bacillus subtilis, and Staphylococcus aureus H (III) which contain α-glucosyl residues joined to ribitol, and O-alanyl groups probably linked to glucose [73]. Staphylococcus lactis I3 are cultured with glycerol and phosphate N-acetylglucosamine 1-phosphate residues to synthesize *teichoic acid polymer.* Lactobacillus plantarum produced teichoic acids possessing phosphate-sugar linkages [74]. New *amphipathic* polymers are produced from nutritionally variant streptococci (NVS) [75]. Neisseria meningitides group B and C, and Escherichia coli K1 and K92, produced polymers of *sialic acid* [76]. *1, 6-anhydro-muropeptides* are obtained by the enzymic preparation of peptides of Escherichia coli fused with staphylococcal protein A. [77]. *O-specific haptenic polymers* are obtained from Salmonella typhimurium [45].

11.3 Methods to Obtain Bacterial Polymers

11.3.1 Conventional Methods (extraction/isolation)

Various methods are involved in the isolation or extraction of bacterial polymers from various species. However, here we will only be providing information concerning a few common methods used for the isolation or extraction of various polymers.

Exopolysaccharide: Exopolysaccharide [EPS] was produced from thirty strains of lactic acid bacteria (sourdough) of Lactobacillus, Leuconostoc, Pediococcus, and Weissella genera. According to the reported literature [78], briefly stated, all cell colonies were grown on agar plates containing modified MRS medium with 40 g/L sucrose. Cell suspension was prepared in MRS broth and the strains were allowed to grown for 24 h at 30°C. The cells were harvested from 0.5 ml by centrifugation (3260g, 5 min), washed with 1mL of sterile water and re-suspended in 0.2mL of sterile water of which 2 µL of the bacterial suspension was spotted on Rogosa and Sharpe (MRS) and MRS-sucrose agar medium and allowed to grow for 24 to 48 hours at 30°C. Slimy colonies were developed which produced EPS. The detailed EPS production, purification, composition, and characterization can be obtained from earlier reference [78].

Heparosan: As reported [6], heparosan, a polysaccharide, was isolated from Pasteurella multocida Type D cells which were grown in synthetic media at 37°C for 24h. The grown cells were harvested by centrifugation and extracted with chloroform. Ultrafiltation and ion exchange chromatography was used for removal of the cells (contaminants) and the small molecules to obtain anionic heparosan polymer of molecular weight in the range of 200–300 K Da [6]. There is great demand for heparin-like polymers in pharmaceuticals; hence, researchers have developed *in vitro* synthesis of heparosan using recombinant Pasteurella multocida heparosan synthase PmHS2 according to the procedure reported [79].

Glycoprotein: Glycoprotein, a square surface (S)-layer glycoprotein lattice, is present on the cell surface of Aneurinibacillus thermoaerophilus DSM 10155. This polymer was isolated according to the reported procedure [26]. For example, A. thermoaerophilus was raised in a 15 liter Bilostatas E fermenter under steady conditions (aeration rate, 6 L/min, dilution rate, 0.3/h, and pH 7.0 ± 0.15, temp 55 °C). Cells were reaped by centrifugation and suspended in 250 ml of 50mM Tris buffer of pH 7.2, which was followed by gentle stirring and centrifugation. The cell pellets were washed six times with Tris buffer and the collected supernatants containing S-layer glycoprotein which was lyophilized. This was solubilized at a concentration of 10 mg/ml in 50 mM Tris buffer, 5 mM EDTA, pH 7.2, containing 40% $(NH4)_2 SO_4$ which was centrifuged to collect supernatant to obtain S-layer glycol protein. This was purified by using column chromatography. Along with S-layer glycol protein, secondary cell wall polymers-peptidoglycon complex were also isolated, purified, and characterized by NMR [26].

Lipopolysaccharide: O3 polymer is one of the lipopolysaccharide [69]. The lipid free cell walls of Pseudomonas cepacia are subjected to mild acid hydrolysis in the presence of sodium dodecyl sulphate. Two polymeric fractions, Fla and Flb, were obtained which were purified by chromatography of the water-soluble products on Sephadex G-50. Fla (8%) is high molecular weight lipopolysaccharide, which gave matching elution profiles for carbohydrate and phosphorus containing un-degraded lipopolysaccharide. However, the ^{1}H NMR of Fla was almost indistinguishable from the polymer fraction Flb (yield, 33%). Upon acid hydrolysis Flb D-ribose (28.2%) and 2-amino-2-dcoxy-D-galactose (35.7%) were produced. A regular disaccharide repeating-unit for the polymer was evident from its NMR spectra. O5 polymer (yield, 21%) of lipopolysaccharide was also extracted from the cell walls of Pseudomonas cepacia. Mild acid hydrolysis followed by gel-permeation chromatography resulted in water-soluble products of phosphorus-free polymeric fraction (yield, 39%). Upon total acid hydrolysis, the polymer produced L-rhamnose (23.6%) and an amino sugar (21%).

In another reported method [80], exopolysaccharide-producing microorganisms (Micro bacterium and bacillus species) were isolated from leaves of sea grass, copper coupons, and rock samples from the rocky intertidal shore of the southern Gulf of Mexico. They were carefully washed with sterile seawater to detach the bacteria and were suspended in 10ml of sterile seawater. The suspended bacteria were also diluted and plated on marine agar, incubated at 30°C for 5 days, and checked with a microscope for the growth of uncontaminated colonies in order to isolate pure exopolysaccharide-producing bacteria. 34 strains were screened for producing EPS. The strains were grown in marine agar plates (MA) as well as in marine broth (MB).

MB recorded good growth of bacterial isolates; hence, EPS production in large quantities was carried in MB. The procedure in brief is explained as follows: In 1 L Erlenmeyer flasks containing 500 ml of MB amended with glucose (30 g/L), 50ml of bacterial species was inoculated and fermented at 30°C for 48 h for bacterial growth. The bacterial growth was determined spectrophotometrically at 520 nm from aliquots that were removed at regular intervals. After exponential growth (24–26h), the culture broths were heated at 100°C for 15 min to inactivate the enzymes capable of degrading the polymer. EPS-producing cells were centrifuged at 4000 g for 30 min at 4°C, and filtered through 0.47 μm diameter HVLP polyvinylidene fluoride filters to obtain EPS, which was purified by precipitation.

Polyhdroxybutyrate: Fifteen bacterial colonies of Pseudomonas pseudomallei were screened due to their ability to produce PHB [81]. These were isolated and preserved in suitable agar medium. Later, the culture medium was optimized for conditions with different carbon, nitrogen, and vitamin content in liquid synthetic medium for good yield of PHB. For large-scale production, the researcher prepared a 24h-old-culture in nutrient broth medium at 300°C. Later this culture medium was transferred to a 500 mL nutrient bath and incubated at 300°C while shaking (20 strokes/min) for 24h. At a later time, the cells were harvested by centrifugation at 8000 rpm for 12 min at 40°C. The harvested

cells were washed and re-suspended in 500 mL of biodegradable polymer production medium, and then were incubated while shaking (20 strokes/min) for 48h at 30°C. After the incubation period, the cells were centrifuged at 8000 rpm for 12 min at 30°C and washed with sterile water. This procedure was repeated twice and the pellets were collected and dried to a constant weight at 60°C. PHB is extracted from the pellets as follows: Briefly stated, the pellets of the cells were crushed and the mass of crushed cells were vigorously shaken with chloroform and allowed to settle. The supernatant was carefully decanted and evaporated to obtain PHB (Figure 11.3 shows bacterial colonies on the agar plate). The polymer was characterized by Gas Chromatography-Mass Spectroscopy technique.

Generally speaking, several bacterial species produced short chain length–medium chain length (SCL-MCL) polyhydroxyalkonates [82]. A new bacterial strain, Pseudomonas aeruginosa was identified which produces a co-polymer consisting of SCL, 3HA units of 3-hydroxybutyrate (3HB), 3-hydroxyvalerate (3HV). In this study, researchers made an attempt to increase the production of these hydroxyalkonates. In brief, Axenic cultures of P. aeruginosa Microbial Type Culture Collection (MTCC) 7925 were grown in a mineral salt medium of pH 7.5 at 30°C while stirring at 200 rpm. The mineral salt medium composition was prepared as reported [82]. The medium was deficient of phosphorus and nitrogen. The cell growth was recorded by evaluating the optical density of the culture broth at 600nm, and the dry cell weight was determined following the reported method [82]. Production of PHA was studied under the impact of various carbon sources. The cells were centrifuged to obtain the pellet of Pseudomonas aeruginosa which was dried to a constant weight, and

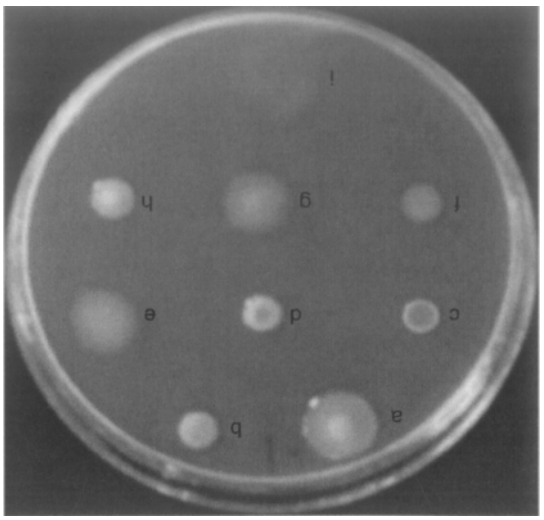

Figure 11.3 Bacterial colonies on agar plates.

the SCL-LCL (PHA) was extracted in hot chloroform and precipitated in cold diethyl ether. The precipitate was centrifuged at 11000 g for 20 min, washed with methanol and dissolved again in hot chloroform. The concentration of the obtained product (SCL-LCL) was estimated following the propanolysis reported method [82].

Polyhydroxyalkonates obtained from bacterial species are in high demand owing to their excellent properties. However, since their production is limited and expensive, studies are being carried out to synthesize polyhydroxyalkonates by my means of chemical modifications and genetic engineering. For example, the low and high molecular weight poly (9-hydroxynonanoates) was synthesized in the laboratory according to the reported procedure [83]. In brief, high molecular weight poly(9-hydroxynonanoates) was prepared according to reported procedure; the pure methyl 9-hydroxynonanoate of molecular weight 188.26 involves several steps such as ozonolysis of oil, reduction and preparation of methyl esters, and purification. Transesterification of methyl 9-hydroxynonanoate was carried out to obtain poly (9-ydroxynonanoates).

11.3.2 Biosynthesis Methods

The industrial and medical requirement for bacterial polymers in large quantities is the basis for enzymatic and genetic engineering approaches which are economically viable and efficient to produce. In addition, for high applicability, tailor-made polymers are also synthesized. According to published reports [84], it is a known fact that in the biosynthesis of bacterial polymers, activated polymer precursor is synthesized using polymer-specific biosynthesis enzymes such as pyrophosphorylases and dehydrogenases. Various bacterial species were enzymatically engineered (metabolic engineering) to produce corresponding polymers. The different polysaccharides that were enzymatically engineered were glycogen (glycogen synthase), alginate (glycosyltransferase), xanthan (xanthan polymerase), dextran (dextransucrase), curdlan (curdlan synthase), gellan (gellan synthase), colanic acid (colanic acid polymerase), K30 antigen (polysaccharide polymerase), cellulose (cellulose synthase), and hyaluronic acid (hyaluronan synthase). As reported [84], polyamides, polyesters, and polyanhydrides were also biosynthesized enzymatically. Some examples of polyamides are as follows: Cyanophycin granule peptide (cyanophycin synthetase), poly-γ-glutamate (poly-γ-glutamate synthetase), ε-poly-l-lysine (ε-poly-l-lysine synthetase), polyesters such as polyhydroxyalkonates (polyhydroxyalkanoate synthase), and polyanhydrides such as polyphosphate (polyphosphate kinase). A detailed protocol of the biosynthesis of various bacterial polymers can be obtained from reported literature [84]. Biosynthetic (i.e. genetic and enzymatic methods) pathways involved in the production of biodegradable aromatic poly-(3-hydroxyphenoxyalkanoates) by Pseudomonas putida is represented in Figure 11.4.

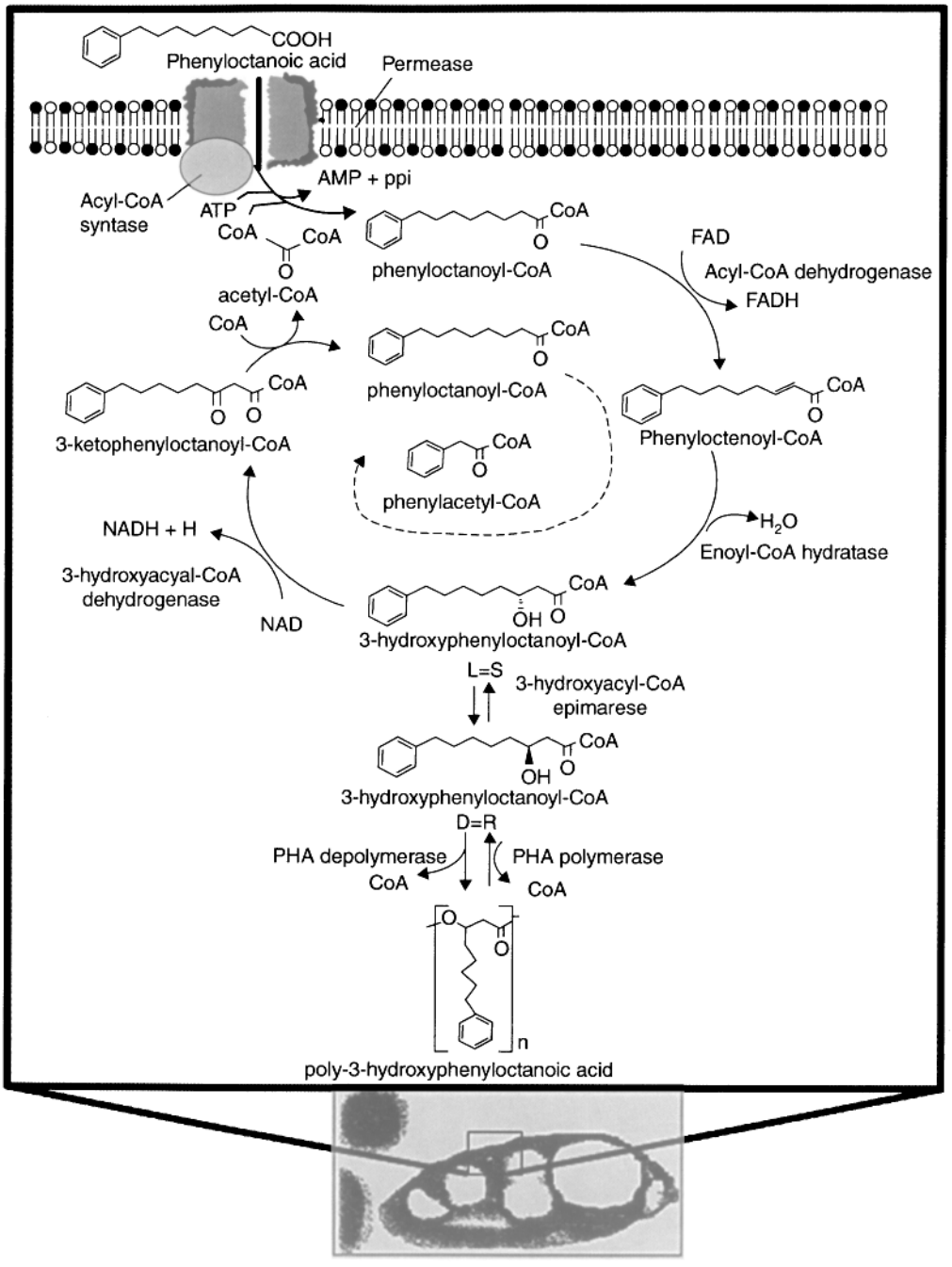

Figure 11.4 Biosynthetic pathways in production of biodegradable aromatic plastics from Pseudomonas putida.

11.4 Tailor-made Methods

Acquiring knowledge on the biosynthesis of genes (genome sequencing, functional genomics, cloning, and characterization) has enabled researchers to understand biosynthesis pathways in organisms [85–88], in order to produce commercially relevant polymers that can replace the petroleum-based, non-degradable polymers. Consequently, this knowledge has been applied to pathway reconstruction and engineering for the improved production and synthesis of tailor-made polymers.

For large-scale recombinant production of bacterial polymers, non-polymer producing bacteria were exposed to biosynthesis pathways. Polymers such as PHA, CGP (cyanophycin granule peptide), HA (hyaluronic acid), and PGA [poly-γ-glutamate] were produced by these methods [89, 85–96]. For example, recombinant E.coli [89] was fermented for the large-scale production of PHA [89]. In addition the PHB biosynthesis genes of Ralstonia eutropa were harbored in E.coli to produce polymers such as PHA composed of (R)-3-hydroxybutyrate and (R)-3-hydroxyvalerate and/or (R)-3-hydroxyhexanoate which showed preferable properties for use in industrial applications [97–99, 85–96].

Recently, new unnatural polymers, including polythioesters and lactate-based polyesters, were produced in recombinant E. coli using biosynthesis pathways [100–102]. New polymers of homopolythioesters with unique properties were produced by recombinant E.coli by utilizing genes encoding phosphotransbutyrylase (Ptb) and butyrate kinase (Buk1) from Clostridium acetobutylicum and the promiscuous PHA synthase from Thiocapsa pfennigii [100]. Similarly, poly(lactic acid) was produced by recombinant E. coli by harboring the genes encoding engineered propionate-Coal transferees and PHA syntheses (Pac). In addition to biosynthetic pathways, the studies on the biosynthesis of new enzymes and polymer modifying enzymes, along with an increased understanding of reaction mechanisms including structure function relationships, has enabled researchers to produce new, tailor-made polymers [103, 104, 105, 101, 106]. Apart from biosynthesis pathways, and engineering of key enzymes, random mutagenesis and site-directed evolution are also valid strategies towards the development of polymer production strains [107, 108–111].

The cell as a biosynthesis machine can use cheap carbon sources (waste products) as precursor substrates to produce bacterial polymers. However, the *in vitro* synthesis of biopolymers requires costly purified key enzymes and precursor molecules such as ATP, coal, coal hoisters, and nucleotide sugars or sugar acids to synthesize polymers such as PHA, cellulose, alginate, and PGA. Consequently, these polymers have limited commercial applicability due to their very high production costs. It is estimated the production of PHB by *in vitro* synthesis would amount to a cost of around US $286,000 per gram of PHB; whereas, bacterial production of PHB was estimated to cost about $0.0025 per gram of PHB, and this is still 5–10 times as expensive to produce as the respective petroleum-based polymers.

One of the more recent and exciting developments is the production of PHB granules by the recombinant bacterial cell as tailor-made functionalized micro- or nano-beads in which specific proteins attached to the PHA core have been engineered to display various protein functions.

Engineered PHA beads were utilized in high-affinity bio-separation [112–114], enzyme immobilization [115], protein production [116], diagnostics [117], and as an antigen delivery system [118] which is currently being commercialized [98, 69]. Poly [(*R*)-3-hydroxyalkanoates] (PHAs) biopolymers can be stored by bacteria, and are currently receiving much attention because of their potential as renewable and biodegradable plastics. The best known representatives are poly (hydroxybutyrate) and its copolymers with hydroxyvalerate, which have been commercialized under the trademark Biopol™.

In addition to these rigid materials, the elastomeric medium-chain-length PHAs produced by fluorescent Pseudomonas are now emerging which can be cross-linked by conventional techniques to yield a biodegradable rubber. A typical application of this material would be a paper coating.

Medium-chain-length polyhydroxyalkanoates (MCL-PHAs), which have constituents with a typical chain length of C6-C14, are polyesters that are synthesized and accumulated in a wide variety of Gram-negative bacteria, mainly pseudomonas. These biopolyesters are promising materials for various applications because they have useful mechanical properties and are biodegradable and biocompatible. The versatile metabolic capacity of some Pseudomonas spp. enables them to synthesize MCL-PHAs that contain various functional substituents. These MCL-PHAs are of great interest because these functional groups can improve the physical properties of the polymers, allowing for the creation of tailor-made products. Moreover, some functional substituents can be modified by chemical reactions to obtain more useful groups that can extend the potential applications of MCL-PHAs as environmentally friendly polymers and functional biomaterials for use in biomedical fields. Although MCL-PHAs are water-insoluble, the microorganisms that produce extracellular MCL-PHA can degrade them. Depolymerase MCL-PHA-degraders are relatively uncommon in natural environments and, to date, only a limited number of MCL-PHA depolymerases have been investigated at the molecular level. All known MCL-PHA depolymerases share a highly significant similarity in amino acid sequences, as well as several enzymatic characteristics [119]. Metabolic pathways for MCL-PHA biosynthesis is shown in Figure 11.5.

Extra cellular polymeric substances (EPS) produced by microorganisms are a complex mixture of biopolymers primarily consisting of polysaccharides, as well as proteins, nucleic acids, lipids, and humic substances. EPS make up the intercellular space of microbial aggregates and form the structure and architecture of the biofilm matrix. The key functions of EPS are comprised of the mediation of the initial attachment of cells to different substrate, and protection against environmental stress and dehydration. The latter has a profound impact on an array of biomedical, biotechnical, and industrial fields including pharmaceutical and surgical applications, food engineering, bioremediation,

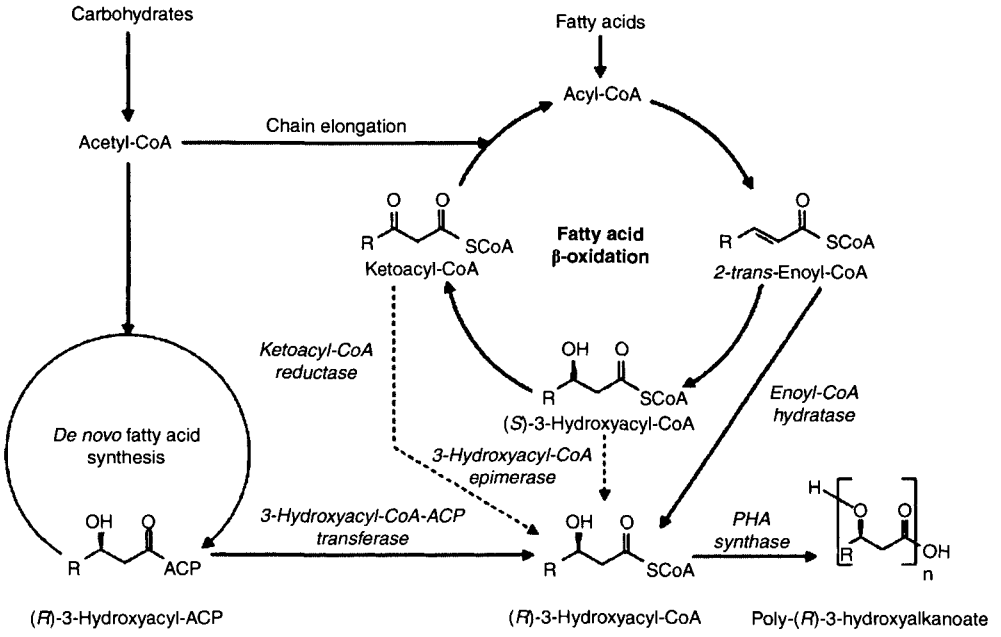

Figure 11.5 Metabolic pathways for MCL-PHA biosynthesis.

and biohydrometallurgy. Finally, a range of novel techniques can be used in studies involving biofilm-specific polysaccharides.

11.5 Applications

Bacterial polymers obtained by any form (e.g. extraction, biosynthesis methods, tailor-made synthesis) are used for various industrial, agricultural, and biomedical applications [120]. The details of the applications are listed in Table 11.1.

11.5.1 Biomedical Applications

Chitin and its derivatives have been used as drug carriers and anti-cholesterolemic agents, blood anticoagulants, antitumor products, and immuno-adjuvants [121,122]. More recently some studies have shown the anti-oxidative and radical scavenging activities of chitosan obtained by bacteria. As a matter of fact, a primary actor in various degenerative diseases as well as in the normal process of aging is oxidative stress, induced by oxygen radicals [123, 124]. Chitin, collagen, and poly-L-leucine have been used to prepare skin substitutes or wound dressings [125]. Alginate gels have been extensively used in controlled release drug delivery systems [126]. Herbicides, microorganisms, and cells have been encapsulated by alginates.

PHB and PHBV are soluble in a wide range of solvents and can be processed in various shapes. Nevertheless, as PHB is brittle, its application in biomaterials is limited. Because PHBV is less brittle it is potentially usable. In addition, PHBV has the unique property of being piezoelectric. It is used in applications where electrical simulation is applied [121]. PHB has the advantageous property of degradability in D-3-hydroxybutyrate, a natural constituent of human blood. As a consequence, PHB is suitable for biomedical applications. It is used in drug carriers and tissue engineering scaffolds [127, 128, 129]. The perspective area of PHB application is in the development of implanted medical devices for dental, craniomaxillofacial, orthopaedic, hernioplastic, and skin surgery. A number of potential medical devices that have been developed on the basis of PHB are bioresorbable surgical sutures [130, 131], biodegradable screws and plates for cartilage and bone fixation, biodegradable membranes for periodontal treatment, and surgical meshes with PHB coating for hernioplastic surgery [132] and wound coverings [133].

An improvement of medical devices based on bacterial polymers by the encapsulation of different drugs, opens up the wide prospects in applications for these new devices with pharmacological activity in medicine. PHB polymer was used as a drug delivery matrix for sustaining the release of various drugs such as dipyridamole [DP], indomethacin and antibiotics (rifampicin, metronidazole, ciprofloxacin, levofloxacin), anti-inflammatory drugs (flurbiprofen, dexamethasone, prednisolone), and antitumor drugs (paclitaxel) [132].

Development of therapeutic systems of sustained drug delivery on the basis of microspheres and microcapsules from biodegradable polymers is a new and promising trend in the modern pharmacology. For example, PHB microspheres loaded with DP were developed and the release mechanisms were studied. The coefficient of the release diffusion of DP extensively depends on the diameter of microspheres. But it is possible to produce a system with prolonged uniform drug release that is important for producing therapeutic systems with adjusted drug dosing. For example, the sustained release of DP from PHB microspheres occurred with almost constant rate for more than1 month.

PHB microcapsules with encapsulated model drug – methylene green have already been reported. The prospects for development and investigation of systems with biological activity based on microcapsules from polyhydroxyalkanoates look bright.

Besides the application of PHB for producing medical devices and systems of sustained drug delivery, PHB can be used for producing systems of sustained release of enzyme activators or inhibitors for the development of physiological models. PHB with its minimal adverse inflammatory tissue reaction under implantation is a perspective tool in the design of novel physiological models of prolonged local enzyme activation or inhibition *in vivo*. A system of sustained nitric oxide (NO) has been developed for donor delivery on the basis of PHB. This system can be used for investigating prolonged NO action

on normal tissues of blood vessels *in vivo*. The development of an *in vivo* model of prolonged NO local action on vascular tissues is a difficult problem, because NO donors deliver NO only for a few minutes. A model of prolonged local NO action on the appropriate artery on the basis of PHB loaded with a new effective NO donor, FPTO [134], has also been reported. It has been shown that FPTO-loaded PHB cylinders can release FPTO (and consequently NO) for up to one month in a relatively constant rate. FPTO-loaded PHB cylinders with sustained FPTO delivery were implanted around the left carotid artery of Wistar rats; pure PHB cylinders were implanted around the right carotid artery as a control. On the first, fourth and tenth day after implantation the arteries and cylinders have been isolated. At the present time, research into enzyme expression and activity in isolated arterial tissues is being carried out [135].

11.5.2 Industrial Applications

Despite several decades, on and off, of research on PHAs and 20 years of intense industrial interest, PHAs still appear to be far removed from large scale production. As of this writing, two developmental programs on these biopolymers are receiving attention, namely: (1) A joint program by the Proctor & Gamble Co. and Kaneka Corp. on a family of short and medium chain copolymers, especially on poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), and (2) a program at Metabolix, Inc. on PHAs for medical applications. The lack of commercialization of the initially promising bacterial poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymers has been generally attributed to the high cost of investment for fermentation and product recovery processes on a large scale, and to the cost of the substrates. To reduce the later limitation, alternative substrates are receiving much attention, including starch and vegetable oils, but no major breakthroughs in this area have been announced. Nevertheless, in the long run, it is possible that advances in our understanding and control of the genetic pathways involved in the biosynthesis of PHAs in microorganisms and plants, could make the industrial scale production of these biopolymers competitive with oil-based synthetic polymers. PHAs have been used for treating wastewater that contained xenobiotic organisms, and (PHB/polyP) complexes have been used for the removal of metal ions (e.g. Ca_2^+, Na^+, Sr_2^+, and Ba_2^+ etc.).

11.5.3 Food Applications

The polymers such as cellulose, curdlan, xanthan, levan, and dextran that are obtained from the respective bacteria as described in section 10.2 are utilized in various food applications. Cellulose is used in food (nata de coco), and curdlan is used in food as a thickener and gelling agent. Dextran is used in healthy beverages, xanthan is used as a rheology modifier and food additive, and levan is used in food biotechnology.

11.5.4 Agricultural Applications

As for the agricultural production of PHAs, the feasibility of genetic pathways has been demonstrated in small plants such as Arabidopsis thaliana [136], but the transfer of this technology into crops such as canola with acceptable production levels is still in the research stage. On the other hand, the chemical modification of medium chain PHAs produced by bacteria is a promising approach to the commercialization of high value polymers for specialty applications [137–139]. Indeed, by either direct bacterial synthesis or by the chemical modification of bacterially produced PHAs, polyesters with more than one hundred different types of repeating units have been identified and characterized [140]. Very recently, it was even found possible to produce a thioester analogue of the PHAs with bacteria, so it is apparent that there is still much more to be discovered about the synthesis of bacterial polyesters.

11.6 Conclusion and Future Prospective of Bacterial Polymers

Currently cellulose (exopolysaccharide) and polyhydroxyalkonates are the most important bacterial polymers which can be profitably used as polymeric material for industrial and medical applications. But it seems to be particularly difficult to achieve a quantitative upgrading of the corresponding extraction, biotechnology and tailor-made synthesis for substantial production at lower prices. Hence, the exploitation of these polymers in various applications will be very difficult until this situation is resolved. Indeed, much awaited is the development of methodologies to increase production of these polymers at affordable prices for more utility.

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12

Gum Arabica: A Natural Biopolymer

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Abstract

Electroactive polymers (EAP), ion, electron, and mixed conducting, have become a new class of high potential materials for future technologies. Natural biopolymers are found to be superior to synthetic polymers in many respects. Recently it has been discovered that a large class of natural polymers exhibit electroactivity. These electroactive biopolymers may be either ion, electron, or mixed conducting. Gum Arabica is a natural plant that exudates carbohydrate type of gum. Electroactivity and its mechanism in gum Arabica will be the main focus of this chapter. Special emphasis will be placed on the various material aspects of gum Arabica and its complexes and the modification of electroactivity of gum Arabica under the external add-on effect. The potential field of application for gum Arabica, its complexes and nanocomposites in developing ionic devices such as batteries, sensors, biosensors, electronic applications, solar materials, energy storage materials, nanoscience, etc., will also be highlighted.

Keywords: Gum Arabica, natural biopolymer, electroactivity, nanocomposites, solar material

12.1 Introduction

On this blue planet, it is of paramount importance to meet the challenge of balancing the highly-principled desire to improve human welfare with environmental challenges. On the path of development the ever-burgeoning energy requirements for a growing economy and population is a real matter of concern. Survival of mankind is totally dependent on nature's bountiful resources; the incessant demands of development are sharply eroding the fragile ecological balance of the planet we inhabit. In such an environment, biomaterials are a clean, degradable, cost-effective, green source of energy using a self-sustaining, ever-replenishing natural resource that is in harmony with nature. In recent times, soft matter [1] occupies a leading position in material research. Most of

† (This chapter is dedicated to Late Prof. Shyamal Sengupta, Emeritus Professor, Dept. of Physics, Presidency College, Calcutta, India).

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (317–376)

the soft matters are derived from living matter. Biomaterials, especially biopolymers, are playing the most crucial role in soft-matter studies. The aforementioned biopolymer systems are mostly dominated by weak, non-covalent interactions, and fluctuations such as Brownian motion are also present in them. This highly interdisciplinary research has gained tremendous momentum from the viewpoint of material science and modern biology. During the last few decades it has been found that electroactive polymers (EAP) are high potential materials for various applications. The electroactivity of a material refers to the charge transport in an otherwise insulating material. An electroactive material has historically been identified as ion conducting solids. The materials were found to be very useful and application-oriented in the field of electrochemical devices or solid state ionics. Later, however, commonly known polymers in electrical or electronic devices were found to be more important. In general, insulating types of materials or systems showing both electronic and ionic conduction are termed electroactive materials. Electroactivity of insulating/dielectric type materials refers to any type of charge transportation in it. An electroactive material may be of the following type: (i) Electron conducting polymer, (ii) ion conducting polymer, (iii) superconducting polymers/organic semiconductors, (iv) ferroelectric ferromagnetic polymers, (v) liquid crystalline polymers, (vi) biopolymers and biocompatible composites. The conventional EAPs are synthetic organic materials that are insoluble, non-degradable and, in general, toxic in nature. Biopolymers are found to have a greater degree of flexibility and functionality over synthetic polymers. Recently, a substantial number of biopolymers have been identified as electroactive biopolymers (EABP). A brief summary and introductory review of EABPs and their functional aspects may be found in an article review [2]. The known EABPs, such as starch, cellulose, chitosan, pectin, plant gums, etc., are found to exhibit electrical conduction over a wide range of conductivity between 10^{-3} and 10^{-14} S/cm. Also, there are many more that are yet to be investigated. Natural polymers are gradually replacing conventional synthetic polymers because the worldwide scientific community has started having a growing interest in their environmental benefits. These are: i) Biodegradable plastics increase the soil organic content as well as water and nutrient retention, while reducing chemical inputs and suppressing plant disease, ii) the energy it requires to synthesize and manufacture most biodegradable plastics is generally much lower than non-biodegradable plastics, and, iii) biodegradable plastics offer environmental benefits by using renewable energy resources and reducing greenhouse gas emissions.

This chapter presents the outcome of the research done for over a decade pertaining to the study of biomaterials, namely, the investigation and understanding of various physical mechanisms, their potential application, and future direction. The research addressed the basic problems to be resolved regarding the electroactivity in natural gums whose chemical characterization/composition was known. The author along with his student also identified their scope of application. Biomaterials are more compelling than conventional materials due to their non-toxicity, eco-friendly nature, and superior cost-effectiveness.

Biomaterials and the various aspects of their study is an extremely wide field. However, their complex molecular structures have made them unpopular over the last century as a potential field of study. The work presented here is a study on biomaterials from a material science point of view, and its aim is to investigate the electrical, thermodynamic, structural, and electrochemical character of gum Arabica along with its possible real-life application.

The range of biomaterials that were studied was mainly plant gums and their composites. Because of medicinal potential, chemical characterizations of only a few gums are available. It has been established that these gums exhibit electrical properties such as a conducting polymer. The detailed nature of conduction and its various characterizations namely, structural, thermodynamic, and optical, are studied experimentally and analyzed. Some encouraging aspects of the aforementioned study have already been recorded and many more have yet to be received. Just like with the conducting polymers, to a great extent the electroactivity of biomaterials can be tailor-made with the addition of other inorganic and/or organic materials. In this chapter, comprehensive results on the experimental study of the electrical properties of biomaterial complexes are explored. The outcome of different investigations and their details are summarized in the following sections.

12.1.1 Natural Gums, Sources and Collection

Gums are natural plant exudates that have oozed from injured sites on tree barks or on fruits, and have hardened upon exposure to air. In general, gums may be classified into three major classes, natural gums, chemically modified natural gums, and chemically synthesized synthetic gums. Popular natural gums are Acacia Arabica, Acacia Catechu, Mangosteen, Karaya, Ghati, Guar, Tragacanth, and some other unidentified gums. Naturally occurring exudate gums are produced in response to injury, e.g. gum Arabica from *Acacia species*, and extractive gums are extracted from fruits of some plants, e.g. gum Mangosteen from *Garciana Mangosteena species*. Some other exudate gums are: Tragacanth gum from *Asiatic astraglus species*, Karaya gum from *Sterculia*, and Ghatti gum from *Anogeissus latifolia*. It is to be noted that natural gums are not plant latex.

Chemical nature of natural gums: In general, natural gums are complex acidic polysaccharides consisting of salts and sugars such as L-arabinose, D-galactose, L-rhamnose, and D-glucuronic acid complexes with metallic ions such as sodium, potassium, calcium, magnesium, etc. [3]. In fact, the chemical composition in particular is not unique, but rather it may vary with the geographical location of the generic plant. In general, these gums have highly branched structures consisting of different monosaccharide units with different possible variations such as degree of branching, length of branches, and type of chemical linkages, in order to produce numerous possible chemical structures. Therefore, an almost infinite number of structures are possible. The molecular forces acting between different portions of the polysaccharides' molecules and that of the solvent may be hydrogen bonding, ionic charges, or

dipole-dipole interactions type. These forces are responsible for the fascinating physical, chemical, and adhesive properties of natural gums. The extracted raw gum from their natural sources may contain inorganic and organic impurities such as ash, tracer water molecule, heavy metallic ligand, bacteria, etc. A purified protected gum may retain its form over thousands of years.

Solubility: There are two categories of gums, water soluble and the resin type which is insoluble in water. Acacia, Mangosteen, Karaya, Tragacant, etc., gums belong to the former category, and pine gum and gum from *Scriptomaria Japonica* are the later type. Gum from the Acacia family is unique among the natural hydrocolliods because of its high solubility in water. The main water soluble fraction of gum Arabica has arabino-galactan in it. The viscosity of the solution is dependent on the concentration of the gum in solution. The other common gums cannot be dissolved in water at a concentration higher than about 5–7% because of their high viscosities. Gum Arabica, however, dissolves readily in cold water up to a 50% concentration and produces a tacky, but not very viscous, solution. Gum Arabica is insoluble in alcohol and other organic solvents. It is slightly soluble in aqueous alcohol with the solubility decreasing as the proportion of alcohol to water increases to about 60% alcohol, and at that level it is practically insoluble.

Specific gravity, moisture content, hardness and physical appearance: Specific gravity of solid gum Arabica is found to vary between 1.35–1.50. Hardness of solid gums partially depends on the amount of moisture content in it. Solid gums are, in general, amber-like amorphous dried exudates or extracts from its generic plant or part of it. Natural gums are nontoxic, biodegradable, and eco-friendly materials. In most cases they are odorless, colourless (or may have pale colour), and tasteless (e.g. gum Arabica). However, there are gums with colour such as gum Mangosteen (dark tea colour) and gum Ghati (reddish colour). Solid gums are insoluble in oils and in most organic solvents, but dissolve completely or partially in hot or cold water forming a mucilaginous solution or suspension. Surface tension of water decreases with the addition of gum Arabica. The freezing point of gum Arabica solutions decreases with the increase of its concentration.

Rheological Nature: Gum Arabica is a polyelectrolyte soluble in water over a wide range of concentration; below 40% concentration pseudo-plastic characteristics are observed, denoted by a decrease in viscosity. With increasing temperature the relative viscosity and density of gum Arabica solution decreases.

12.2 Chemistry of Gum Arabica

Chemical Character: Most of the commercial gum Arabica is derived from Acacia Arabica from the 500 species of Acacia trees distributed throughout the tropical and subtropical areas of the world. One of the oldest known adhesives and thickening agents, it is found in nature as a neutral or slightly acidic salt of a complex polysaccharide [4–10] containing tracer amounts of calcium, magnesium and potassium cations. Molecular weight determination gives different

results by various methods; 2×10^5 to 2.5×10^5 are found from osmotic pressure data, and 2.5×10^5 to 3×10^5 by sedimentation and diffusion. Values more than 10^6 have been obtained by light scattering study. Some studies also suggest that in gum Arabica, macromolecules are in the shape of short, stiff spirals with numerous side chains. The length of the coil is 1050 nm. The aqueous solution of the gum is acid with a pH range of 2.2 –2.7. This natural acidity promotes a slow autohydrolysis and yields L-arabinose which is the first manose liberated from the macromolecules followed by L-rhamnose, D-galactose and D-glucuronic acid. Macromolecules are branched and the various anhydromonoses are linked by 1, 3, 1, 4 and 1, 6 glucosidic bonds. The complex structure contains chains of L-arabinose (24%), L-rhamnose (7%), and L-galactose (67%) interlinked with D-glucuronic acid (~2%) unit. Gum Arabica solutions are levorotatory (specific rotation –20°–30° with sodium light). The chemical structure of gum Arabica molecule may be found in reference [9].

12.2.1 Potential Use as Material

Following is a list of the many possible uses of gum Arabica: (i) In dairy products it is used as a stabilizer in frozen ice creams, ices, etc. because of its water absorbing properties. (ii) In the brewing industry it is a foam stabilizer for beer as well as other beverages. (iii) In confectioneries it prevents the crystallization of sugar. (iv) In the pharmaceutical industry it is used for treatment of low blood pressure caused by hemorrhage or surgical shock, and is also used as a binder in tablets, as an emollient, and a demulcent in cough drops and syrups. According to a recent report [11], gum Arabica has been found to act as an antioxidant, and also protects against experimental hepatic-, renal- and cardiac toxicities in rats. (v) In cosmetics it stabilizes emulsion, increases viscosity, adds a smooth feel to the skin, and forms a protective coating in lotions and protective creams. It is also used as a foam stabilizer in the production of liquid soap. One advantage of gum Arabica in cosmetics is its non-toxicity and its comparative freedom from dermatological and allergic reactions. (vi) In adhesives and as a binder. It is considered to be a safe, simple adhesive for use in paper products. Nigeria produces four different grades of Acacia gum in commercial quantities. In a recent study [12] it was found that the physical and chemical properties of Nigerian *Acacia species* showed its viability for foundry sand binding. (vii) In the textile industry it is widely used in the printing formulations for fabric design. (viii) In the production of inks. It is a constituent of many special purpose inks due to its excellent protective colloid properties.

12.3 Electroactivity of Gum

In this chapter the emphasis will be on electrical transport as the hallmark of electroactivity. Electrical conductivity of polymers is a material property which may vary over a wide range. Biopolymers are a subclass of organic conductors. The electrical conductivity in what is otherwise a biopolymer originates from

motion of either electrons or ions or both. The electrical conductivity of biopolymer is given by:

$$\sigma = \Sigma\, q_i\, n_i\, \mu_i \tag{12.1}$$

q_i, n_i and μ_i are the respective charge, concentration, and mobility of the ith species of conducting carrier. The carrier mobility is the carrier drift velocity in the field direction per unit electric field. Electronic conduction in a biopolymeric system is possible where unsaturated or π-type chemical bond exists. The band theory also explained electron and hole conduction in a polymeric system. A comprehensive discussion along with various aspects of electrical conductivity can be found in literature [13]. Explanation of ionic conduction in an ionic solid may be realized as follows: In the absence of an electric field, the thermally generated Frenkel or Schottky defects are mobile and when a field is applied the defects will drift with their corresponding mobility and give rise to electrical conductivity. In ionic solids, two types of Frenkel defects are possible, cations and anions. Due to a difference in size of both ions, the energies required to put either of them into interstitial positions differ considerably. Hence, electric current is carried predominantly by either of the ions. In general cations are more mobile than anions.

12.3.1 Ionic Conduction in Electroactive Material

It has been observed that the gross electrical conduction mechanism in natural gum Arabica has a resemblance with that of ion conducting doped polymers. The ionic conduction and ionic conductors is a self-content subject; a brief overview of the latter class of materials is summarized in the following subsections. Ionic compounds may be divided into three groups. (i) A perfect crystal of an ionic compound is an insulator with a residual ionic conductivity lower than 10^{-10} S/cm. (ii) Ionic conductors in which the presence of thermally generated Frenkel defect or Schottky defect pairs or disorder produces ionic transport leading to conductivity up to 10^{-5} S/cm. Again defect concentration classifies point defects into two categories. Dilute point defects (~10^{18} defects /cm^3) and concentrated point defects (~10^{18-20} defects /cm^3). The activation energy E_a varies between 0.6 – 1.2 eV. (iii) In liquid-like molten sub-lattice the number of ions of a particular type (~10^{22} defects /cm^3) are less than the number of available sites in their sub-lattice and the entire ions "hop" from one position to another with lower activation energy for the defect process. Loosely packed structure facilitates dynamic disorder and diffusion. The different types of ion conducting materials are classified according to the natural electrical conduction in them and are as follows: An ionic solid whose ion transference number is greater than 80% is called a superionic solid. The ion transference number (τ) is defined as the ratio of ionic conductivity to total electrical conductivity of a solid. The total electrical conductivity of ionic substances consists of contribution from ions and electrons (via defect). It follows from the above discussion that both concentrated point defect type and molten sub-lattice type

solid represent superionic conductor [14]. There exists no sharp demarcation between superionic conductors and other ionic compound - combination of melt like ionic conductivity ($\sigma \geq 10^{-4}$ S/cm) in the liquid sub-lattice with a low activation energy (~10^{-1} eV) are the hallmarks of the superionic phase. The mechanism of conduction in an ionic or a superionic solid is summarized here.

12.3.2 Conduction Mechanism

Biomaterials, like gum Arabica, are found to exhibit electrical conductivity by transport of ions in some form of proton. Isolated protons (H^+ ions) cannot present in solids under equilibrium conditions because the small ionic radius (<1.05Å) produce a high charge density which polarize the surroundings, hence, protons are covalently bonded to one or two electronegative atoms/ ions of the surrounding due to its strong polarizing power. The following three different bonding situations for the proton may occur [15]:

i. The acceptor site for the proton may be an ion of the immobile lattice, e.g., a hydroxyl group is formed.
ii. The proton may be attached to a mobile ion, e.g., an oxygen ion hence a hydroxyl ion OH^- is formed:

$$H^+ + O^{2-} \rightarrow OH^-$$

iii. The proton is attached to a mobile molecule such as one or two water or ammonia, thus forming a hydronium/di-hydornium or ammonium ion by the following reaction:

$$H^+ + H_2O \rightarrow H_3O^+,\ H^+ + 2H_2O \rightarrow H_5O_2^+,\ H^+ + NH_3 \rightarrow NH_4^+$$

Proton conduction occurs either by lone proton migration or in general by proton-carried migration, called vehicle mechanism. The lone proton migration or proton translocation or Grotthuss process [14] occurs in oxonium perchlorate and potassium hydrogen phosphate (case i above). Proton carried migration occurs in oxonium β-alumina and hydrogen uranyl phosphate (case ii and iii), H_3O^+ is the mobile species. Solid protonic conductor (SPC) is a material in which protons can be transferred through the solid material and are converted into hydrogen gas at the cathode with the exception of metal hydrides. Hydrogen evolution over long periods of time with a supply of protons in some form at the anode could be taken as proof of protonic conductivity. The conducting species in SPC's are protons, oxonium ions, ammonium ions, hydrazine ions or hydroxyl ions.

12.3.3 Ion Transference Number

The ion transference number of mobile ions (mostly hydronium) in the solid gum Arabica biopolymer is estimated by Wagner's polarization technique [16] using blocking electrodes. The ionic transference number τ_{ion} is found [17] to

be 0·96. Hence, the observed specimen of gum Arabica exhibits ionic character like other solid electrolytes and current occurs mostly due to the motion of some of the conducting ion species which is discussed in the next section.

12.3.4 Conducting Ion Species in Gum Arabica

The observed electrical conduction in gum Arabica is like that in inorganic salt doped synthetic polymers. From the view point of material science, it is essential to have knowledge on conducting carrier species responsible for the ionic conduction phenomenon. The conducting species in solid protonic conductors (SPC) are proton (H^+), hydronium (H_3O^+), hydroxyl (OH^-), ammonium (NH_4^+), or dihydronium ($H_5O_2^+$) ion. These ion conductions exhibit both bulk and surface conductivity in many SPCs [15]. After a careful experiment by an electrochemical process [18,19], it was found that the gum Arabica sample is also a SPC and the conducting ion species in it were hydronium (H_3O^+) ions. After a long time in d.c. electrolysis of the sample using porous Cu electrodes no hydrogen gas was detected [17, 20] at the cathode but a loss of the sample weight was recorded. The mentioned loss of weight of the gum Arabica specimen was exactly equal to the gain of weight by the anhydrous copper sulphate, which is used as a water vapour trap. The experiment was performed in a glass chamber at room temperature for 3 hours at an average electric current of 2·1 mA and the observed loss in specimen weight was 5·0 mg. It was also found that the observed loss in weight was in accordance with Faraday's law of electrolysis with hydronium as conducting species. The weight of the sample (along with the electrodes) used was equal to 1·72 g with cross-sectional area 5 cm^2 and applied potential difference being 70 V.

12.3.5 Carrier Mobility in Gum Arabica

The ionic mobility of mobile species of the gum Arabica specimen was determined by using transient ionic current technique. The sample was subjected to a d.c. electric field across Cu/sample/Cu cell to polarize it. The transient ionic current was then recorded (using a computerized data acquisition system) as a function of time by reversing the polarizer electric field. The variation of transient ionic current as a function of time for the gum Arabica sample is shown in Figure 12.1 [17, 18]. This very simplistic measurement exhibits a single clear peak, which indicates that the current is due to a single ion species, in this case it is hydronium (H_3O^+) and its ionic mobility μ is estimated using the standard formula, $\mu = (d^2 / \Gamma V)$ where d (=1 mm) is the sample thickness, Γ (= 50 sec) the time of flight, and V (= 1·5 Volt) the applied reverse voltage. There exists some uncertainty in the measurement of Γ, which is due to lack of very sharp resolution in the determination of the peak in the measurement. However, the error involved in it does not alter the order of magnitude of the estimated value of μ.The value of the ionic mobility obtained from the measurement using the formula is found to be: ~ $1{\cdot}33 \times 10^{-4}$ cm^2 V^{-1} s^{-1}.

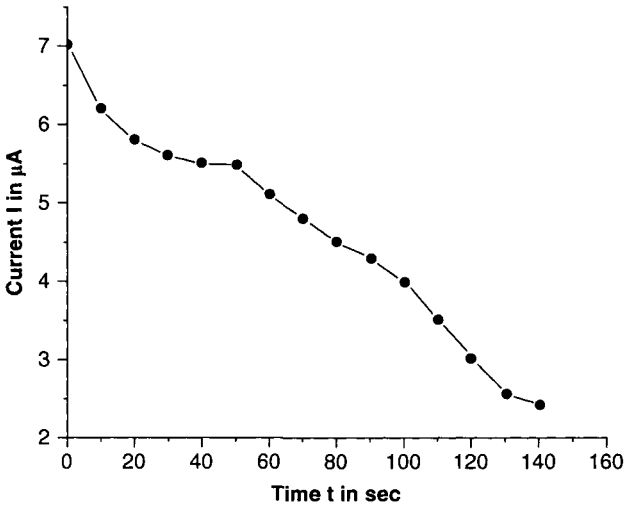

Figure 12.1 Plot of transient ionic current I (in μA) vs. time elapsed t (in sec). Applied Voltage: 1.5 V, Specimen thickness: 0.1 cm, C.S.A: 1 cm^2 [17].

12.4 Method of Characterization

Biomaterials such as natural gums are extracted from living matter. The molecules forming these biomaterials are known to be very complex in nature. Water content in biomaterials is an essential characteristic of them. The water content plays a crucial role in its physical properties like electrical conduction through it. Since these materials are either a covalent or a hydrogen bonded system they cannot be used and tested at temperatures above 120°C. It is apparent, therefore, that not all conventional methods of material characterization can be applied. Thus, as a method of material characterization, some of the conventional methods are used in a restricted way so as to retain the biomaterial characteristics. The characterization method used in the study of natural gum Arabica is summarized in the following sections.

12.4.1 Microscopic Observation

Gum Arabica and its complexes used in the present study contain fine structures which affect their properties. The microscopy may be informative in investigating the molecular domain and block structure in it. The different types of microscopy used in the characterization of gum specimen are: (i) Optical microscopy, where only structures separated about 1μm across can be investigated. (ii) Electron microscopy in the form of transmission electron microscopy (TEM), scanning tunneling microscopy (STM), atomic force microscopy (AFM) and scanning electron microscopy (SEM). The mentioned techniques and tools are able to provide a magnification up to 10^6 and at very high resolution.

12.4.2 Microscopic Observations

The gum Arabica biopolymer must be examined microscopically for verifying its morphology and microstructure. Figure 12.2a and 12.2b shows the SEM photograph of the caste surface of the biopolymers on smooth and scratched substrate respectively. The latter surface was a textured one with grown anisotropy due to cast over scratched substrate. It was taken at a magnification of 10,000, and indicates a domain like microstructure, which is precursor of a growing anisotropy in the specimen [21].

X-ray Diffraction: It is used for the investigation of the microstructure of the matter. This technique is applied to crystal structure determination, chemical analysis, stress measurement, study of phase equilibrium, measurement of particle size, etc. X-rays are electromagnetic waves about 0.1-0.2 n.m. Scattered X-rays from a structure provide information about the details of the structure if the x-ray wavelength is comparable to the interatomic distance in crystals. Considering a particular direction, though each lattice point in the crystal scatters in this direction, the crystal as a whole will scatter strongly in this direction if a large number of scattered rays reinforce one another due to scattering from all structures contained in the sample. This phenomenon is called diffraction. The scattered beams in this direction are completely in phase and hence reinforce each other. In all other directions of space, the

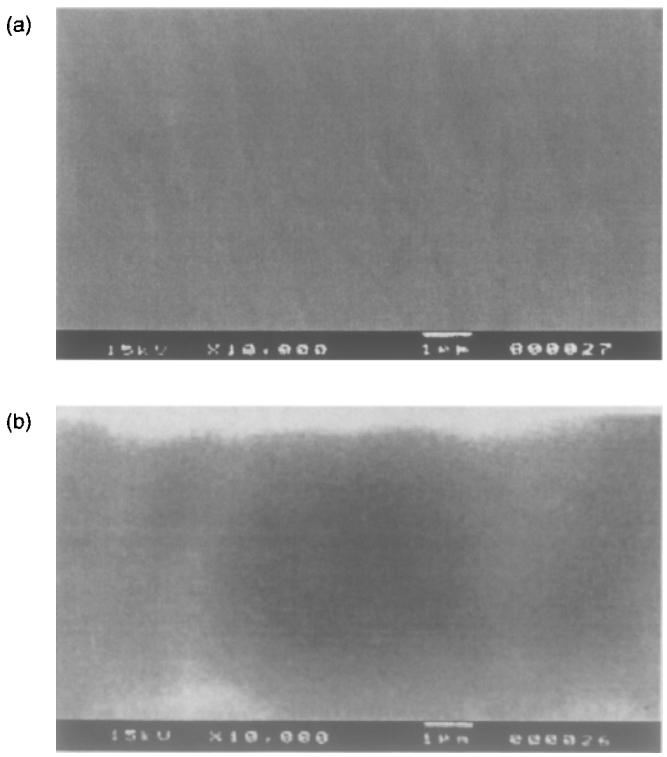

Figure 12.2 (a) SEM picture of untextured surface of Gum Arabica specimen [21]. (b) SEM picture of textured surface of Gum Arabica specimen [21].

scattered beams are out of phase and annul one another. The superposition leading to a XRD spectrum contains all information of the microphase of the specimen.

The XRD pattern of pure gum Arabica powder specimen is shown in Figure 12.3. It shows the overall amorphous nature of gum Arabica. The intensity peak corresponds to $2\theta = 18.893°$ is the peak in Radial Distribution Function (RDF). The Debye-Scherrer equation given in Equation 12.2 is employed to compute coherent length:

$$L = [\ 0.9\ \lambda\ /\ (\ Cos\ \theta_b \bullet \Delta 2\theta_b)\ ] \tag{12.2}$$

where λ is the wavelength of X-radiation (λ = 1.5418 Å for Cu-Kα), θ_b is the glancing angle, $\Delta 2\theta_b$ is the difference in angle at the two ends of full width at half maximum (FWHM). The coherence length is the measure of average grain size in direct space and was estimated in Equation 12.2 as 12.6933 Å.

Figure 12.4 shows the XRD pattern of the crushed pellet of gum Arabica (drawn over a smooth glass surface) measured in the same XRD unit. The XRD pattern provides an interesting feature of intensity distribution. The peak of the distribution shifted considerably in comparison to the one in Figure 12.3. This is an indication of growing anisotropy in specimen due to drawing and casting. The RDF of the pattern shows an increased long range order of molecular distribution in the biopolymer compared to its amorphous state shown by Figure 12.3. The intensity peak of Figure 12.4 corresponds to $2\theta = 19.411°$ and d-space is estimated to be 4.5692 A° using Kα lines.

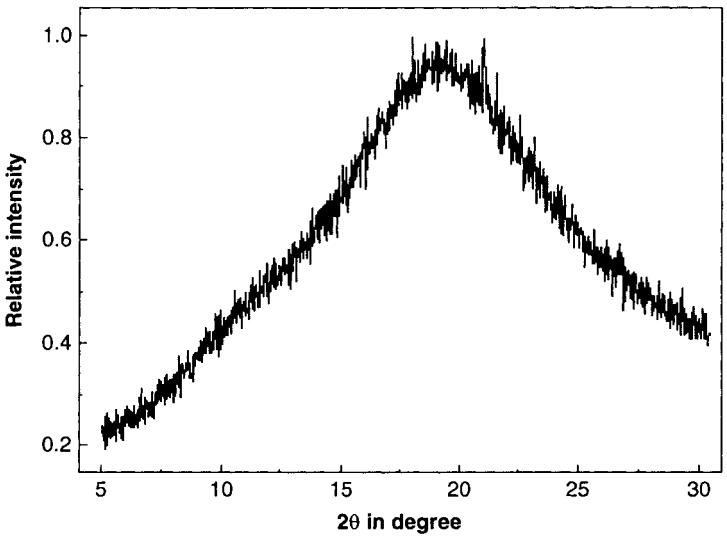

Figure 12.3 Relative intensity vs. 2θ in degree for powdered Gum Arabica sample. Resolution = 0.02 degree; scanning rate = 0.5 s/step [17].

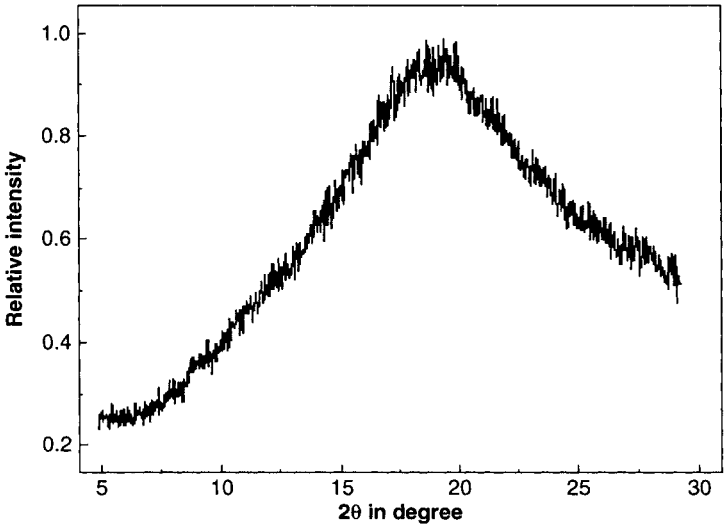

Figure 12.4 Relative intensity vs. 2θ in degree for caste pellet sample of gum Arabica. Resolution = 0.02 degree; scanning rate = 0.5 s/step [21].

12.4.3 Thermodynamic Analysis

Thermodynamic analysis is an important study when information about thermal stability, and structural or phase transformation of material specimen is needed. The principles of different types of analysis of thermodynamic studies are slightly different and those used in the investigation of gum specimens were, (i) Thermogravimetric analysis (TGA), (ii) differential thermal analysis (DTA) (iii) differential scanning calorimetry (DSC). The mentioned instrumentation techniques are applied to investigate water content, phase transformation, and thermal stability of the gum specimens that are analyzed. The TGA result on gum Arabica is shown in Figure 12.5 [17]. It confirms that it is thermally stable up to a temperature of 80°C where it was devoid of its free water by a reversible change. The experimental data indicates 2% free water content in gum Arabica sample. Above the temperature of 120°C, the transparent sample becomes dark grey and losses its electrical conduction property. Carbohydrate type biomaterials (e.g. gum Arabica) never show any glass transition; rather they turned into flakes and finally charred. This irreversible change of gum Arabica is the feature of solid protonic conductor [15] on heating in a water lean atmosphere.

Electrical studies: The electrical analysis on gum Arabica includes a wide range of studies that extract the electroactive nature of gum specimens. Almost each and every electrical technique is based on electrical conductivity measurement. The electrical conductivity of an electrolyte is given in Equation 12.3,

$$\sigma = (d / R\,A) = G\,(d / A) \tag{12.3}$$

where G is conductance (= 1/R, R is the electrical resistance) of the sample of thickness d cm and cross-sectional area A cm^2. The total bulk electrical

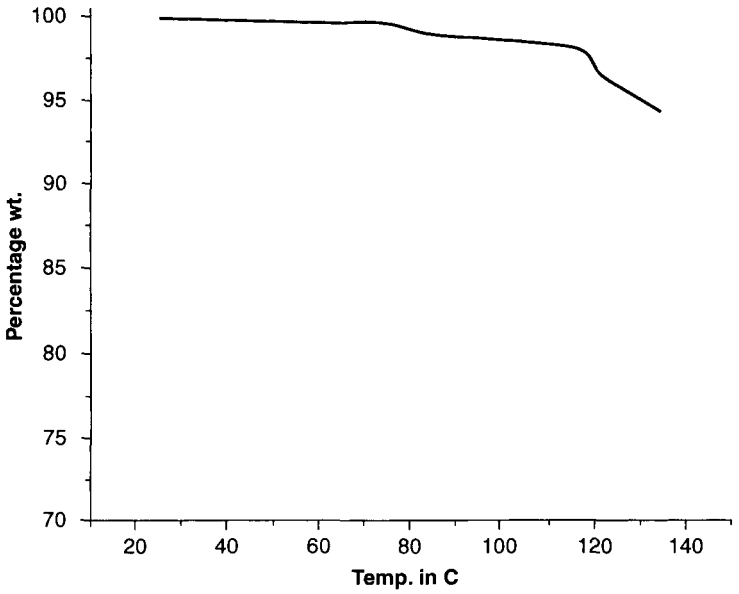

Figure 12.5 Percentage loss in weight (w) vs. temperature (t) in °C (Gum Arabica sample weight=12.9 mg) [17].

conductivity in a material is the sum of electronic and ionic contributions and is given in Equation 12.4.

$$\sigma = \sigma_{electronic} + \sigma_{ion} \tag{12.4}$$

The electronic conductivity is due to contributions from negative electrons and positive electron "holes" and is given in Equation 12.5.

$$\sigma_{electron} = \sigma_e + \sigma_h \tag{12.5}$$

The ionic conductivity is due to both cation and anion species. The cationic transference number $\tau_+ = (\sigma_+ / \sigma_{ion})$. The anionic transference number $\tau_- = 1 - \tau_+$. Different ionic and electronic contributions to the total conductivity can be measured by an electrolysis experiment with the use of selective blocking electrodes which is for blocking all the conducting ion species but the desired one [16, 22].This is a direct current (d.c.) experiment.

Impedance spectroscopy: Impedance spectroscopy describes the response of a circuit to an alternating current or voltage as a function of frequency. In alternative current (a.c.) theory following Ohm's law given in Equation 12.6,

$$E = IZ \tag{12.6}$$

where E is impressed a.c potential difference and Z is defined as impedance, the a.c. equivalent of resistance. The real and imaginary components of complex

impedance Z were estimated from the measured parameters. The measurement was done by an impedance analyzer or LCZ meter. Impedance plot is a popular format for evaluating impedance data, known as the Nyquist plot. This format is also known as Cole-Cole plot or a complex impedance plot. The negative imaginary impedance component Z′ is plotted against real impedance component Z″ at each excitation frequency.

12.4.4 Electrical Polarization and A.C. Conductivity

Following the discussion on ionic conductivity in section 12.1, and protonic conduction in section 12.1.2, it can definitely be seen that overall conduction in gum Arabica belongs to the aforementioned category. The nature of the mentioned conductivity is analyzed from a.c. conduction. In the microscopic level mechanism in the solid, there is a particular pair of states between which jumps take place which are influenced by the electric field. A dielectric material of natural type gum containing permanent dipole moment μ, when sandwiched between two plane parallel electrodes of area A, separation d, the conductivity σ and dielectric constant ε are connected to conductance G and capacitance C by $\sigma = G\,(d/A)$ and $\varepsilon = C\,(d/\varepsilon_0 A)$. In the absence of an external electric field, dipoles are oriented at random and possess only electronic polarizability in the field direction.

An external electric field E orients the dipoles in the direction of the field, creating average total dipole moment along the field direction Total polarization $P(t) = P_e + P_a + P_d\,(t)$. The rate of change in total polarization P (t) gives total electric current through such a specimen in an a.c. experiment. The displacement current thus generated, is related to the total frequency dependent complex permittivity $\varepsilon(\omega) = .\varepsilon'(\omega) + i\,\varepsilon''(\omega)$, $\varepsilon'(\omega)$, and $\varepsilon''(\omega)$ representing dispersion and loss characteristics. The dielectric loss and its frequency dispersion is response dependent. This response involves some rearrangement of the structural units of the polymer and the extent to which changes occur determine the dielectric constant of the biopolymer. Dielectric relaxation in solid biopolymers may have proximity to that in synthetic polymers. In the former there exists multiple relaxation process due to their inherent molecular structure and dipole distribution. The microscopic Brownian motion associated with dielectric relaxation originates from orientation of dipole unit in the solid. The complex character of the relaxation may be analyzed from the results of the experimental variation of imaginary permittivity (ε'') with temperature. An indirect manifestation of the relaxation process is often reflected in non-linear Arhenius character of biopolymer.

If the relaxation is due to a single process of random jumping between two molecular states, the correlation time τ_c is proportional to the average time between jumps; where in the equation $\tau_c = 1/\nu$, ν is the number of jumps per unit time between the two states. An external electric field E orients the dipoles in the direction of the field, creating average dipole moment along the field direction at temperature T. An external alternating field $E(t) = E_0 \exp(j\omega t)$. At low frequencies dipoles have time to orient and are accompanied

by a displacement current, and they contribute their full share to polarization. However, at high frequency the dipoles could not follow the electric field variation and ε approaches to ε_∞.

The dielectric spectroscopy is an essential probe for nondestructive studies required for biomolecule analysis. The study of internal motion/dynamics related to the dielectric relaxation in biomolcules require the coverage of periodic vibration along with other mechanisms, such as diffusion, molecular orientations, and relaxation processes.

Generally speaking, the material application of the electric field follows the relation expressed in Equation 12.7,

$$J(\omega) = \sigma^*(\omega)\, E(\omega) \tag{12.7}$$

where J (ω) is the current density, E (ω) is a.c. the electric field and $\sigma^*(\omega)$ is the complex conductivity. Equation 12.7 may also be expressed in the following form:

$$J(\omega) = i\,\omega\,\varepsilon_0\, K^*(\omega)\, E(\omega) = i\omega\,\varepsilon_0\,(\varepsilon' - i\,\varepsilon'')\, E(\omega) \tag{12.8}$$

where $K^*(\omega)$ is the complex relative permitivity, ε' and ε'' are the real and imaginary part of $K^*(\omega)$, and ε_0 is the permitivity of free space. In the following Equation 12.9, the complex conductivity is given by,

$$\sigma^*(\omega) = \omega\,\varepsilon_0\,\varepsilon'' + i\,\omega\,\varepsilon_0\,\varepsilon' \tag{12.9}$$

where $\varepsilon''/\varepsilon'$ is the dielectric loss factor, which is a measure of energy absorption from the a.c. field. The general behavior of $\varepsilon'(\omega)$ shows that its discontinuities are related to the relaxation and vibration of microscopic constituents of the material and the complex $\varepsilon^*(\omega)$ expressed [10] in Equation 12.10.

$$\varepsilon^*(\omega) - \varepsilon_\infty = (\varepsilon_S - \varepsilon_\infty)/(1 + i\,\omega\,\tau) \tag{12.10}$$

This is known as a Debye dielectric response, where ε_s and ε_∞ are the low and high frequency dielectric constant, and τ is the time constant

Many materials display non-Debye dielectric behavior by a broader asymmetric loss peak. This non-Debye a.c response can be described by a combination of Cole-Cole [23] and Davidson-Cole [24] functions, and an empirical expression proposed by Havrilink-Negami [25]. The natural gum Arabica is found to obey a non-Debye type of response [25, 26] and may be described [27] by the Havrilink-Negami function.

Bulk electrical conduction: The gross feature of the electroactivity of a material is best understood from the a.c. experiment. The variation of complex impedance and loss angle with frequency of the applied voltage was studied for better information about the a.c. conductance of the biopolymer gum Arabica. This measurement was carried out on a gum Arabica sample caste on a plane copper surface between frequency ranges 0.5 Hz – 100 KHz and between

5 Hz – 9.5 MHz by the impedance analyzer. Figure 12.6 is the Cole-Cole plot (Nyquist plot), i.e., the imaginary part of impedance Z″ vs. real part of impedance Z′ is plotted [17] and shown in Figure 12.6 from the measured impedance data calculated using a Fortran program. It shows a depressed semicircular arc within the measured frequency interval. The tail observed at low frequency range is due to the presence of constant phase element. The center of the semicircle is below the real axis and makes an angle ~ 20° with the extrapolated left cross point of the arc at the real axis. The observed single semicircle in Cole-Cole plot indicates the existence of single relaxation process of this SPC. The value of the extrapolated left cross point of the impedance spectra is 310Ω, from which maximum total conductivity calculated is 1.1×10^{-4} S/cm. The extrapolated right crossing point to the real axis corresponds to d.c. resistance of 21,600Ω, from which room temperature bulk conductivity of $1{\cdot}5 \times 10^{-6}$ S/cm^{-1} is calculated. Figure 12.7 shows the Cole-Cole plot of textured gum Arabica specimen by casting on unidirectional scratched copper electrode. Within the 100 KHz limit only part of the semicircular arc is observed. A study of the plot confirms the growth of anisotropy [21] in the specimen.

Frequency dispersion of conductivity: The variation of a.c. electrical conductivity of gum Arabica specimen with applied a.c. frequencies between 1 mHz and 100 KHz at room temperature is plotted in Figure 12.8 [17] The experimental a.c. conductivities are calculated from the measured data, in the following Equation 12.11,

$$\sigma_{ac}(\omega) = (d/RA) \tag{12.11}$$

where R is the real part of the impedance, d is the sample thickness, and A is the cross-sectional area (CSA) of the electrodes. The variation of a.c. conductivity

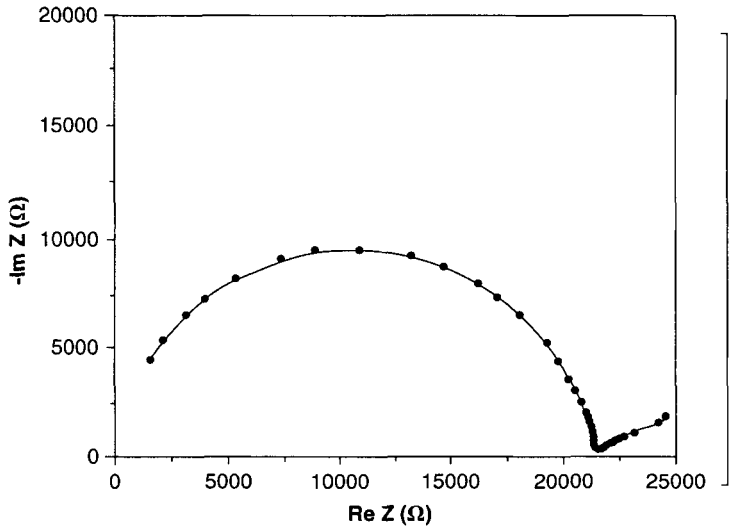

Figure 12.6 Cole-Cole plot of pure Gum Arabica. Frequency range : 0.05 Hz – 100 KHz. Sample specification: thickness d = 0.1 cm; C.S.A = 3 cm^2 [17].

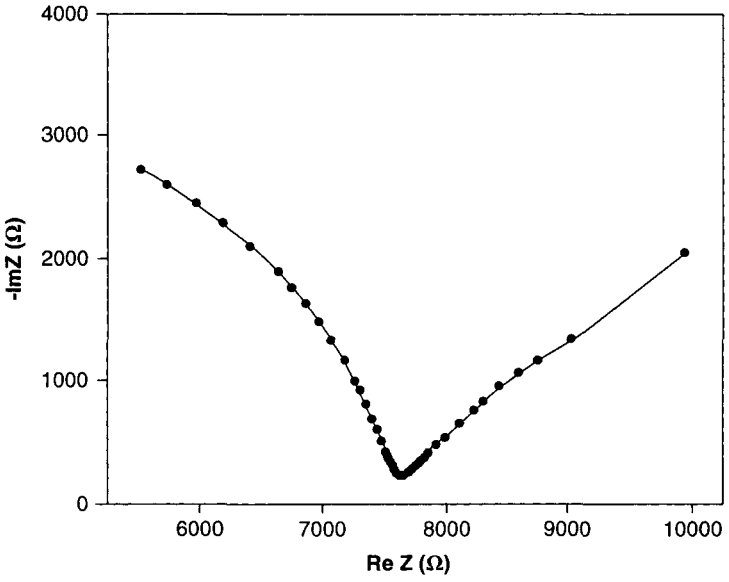

Figure 12.7 Cole-Cole plot of textured Gum Arabica specimen; Frequency range 1Hz – 100 KHz. Textured Specimen thickness = 0.6 cm, C.S.A = 1.6 cm^2 [21].

with frequency ω can be described following Jonscher's power law [28] shown in Equation 12.12,

$$\sigma_\omega = \sigma_{dc} + K\omega^n \Rightarrow log\,(\sigma_\omega - \sigma_{dc}) = n\,log\,\omega + log\,K \qquad (12.12)$$

where, K and n are temperature dependent, frequency independent material parameters. The value of n may be directly estimated from the slope of the above equation. But the graphical nature of Figure 12.8 is not in complete agreement with the above equation. A reasonable value of n found to be 0.98 with value of σ_{dc} obtained from extrapolated σ_ω to zero frequency and found to be ~ 1.2×10^{-9} S/cm and 1.5×10^{-6} S/cm respectively. At low frequency the value of the exponent reduces to 0.8. The dielectric response corresponds to the non-Debye theory of dielectric loss.

Figure 12.9 shows the variation of a.c. electrical conductivities of gum Arabica specimen with applied a.c. frequencies with temperature as parameter. The observed nature of variation is similar to that of other synthetic conducting polymers.

Figure 12.10 shows the frequency variation of conductivity on textured gum Arabica specimen which again shows a deviation from total amorphous nature. From the analysis of experimental results it has been found that the measured transverse d.c. electrical conductivity (measured in direction perpendicular to textured direction) was about 100 times more than that of an amorphous specimen, and the value of (σ_{dc}) obtained from this present measurement was estimated to be ~ 10^{-5} S/cm. Overall bulk conductivity of textured specimen was observed to have decreased with respect to that of the untutored specimen.

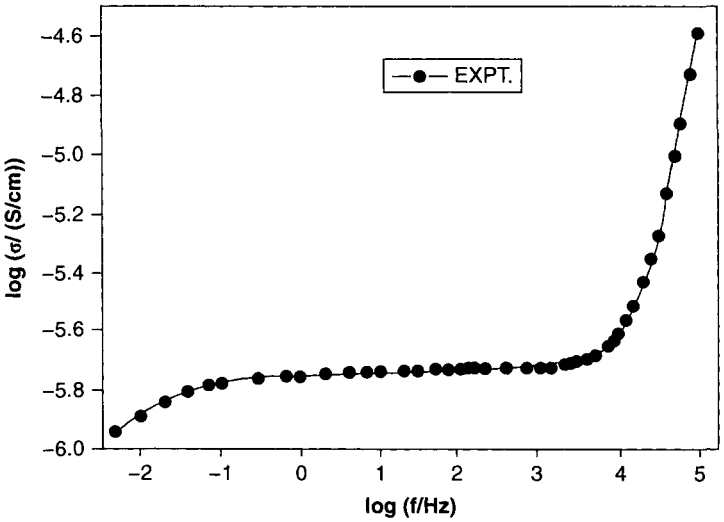

Figure 12.8 Impedance spectrum of pure Gum Arabica, Sample specification : thickness d = 0.1 cm; C.S.A = 3 cm^2 [21].

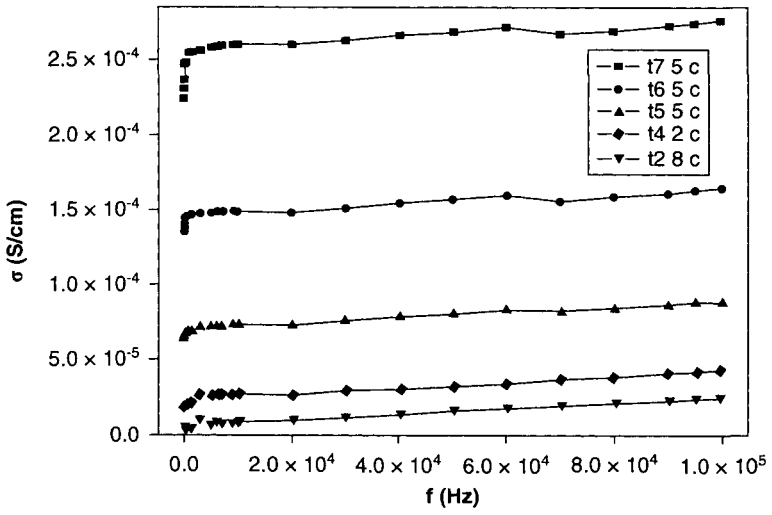

Figure 12.9 Impedance spectrum of pure Gum Arabica with temperature as parameter (with s specification in Fig. 12.8) [10].

Figure 12.11 shows the modulus spectra obtained from the data recorded by the impedance analyzer between the frequency range 5 Hz – 9.5 MHz, and the peak of the curve does not coincide to that of impedance spectra [21]. Therefore, it may be concluded that the dielectric response corresponds to the non-Debye theory of dielectric loss. This indicates the complete amorphous behaviour of powdered gum Arabica specimen.

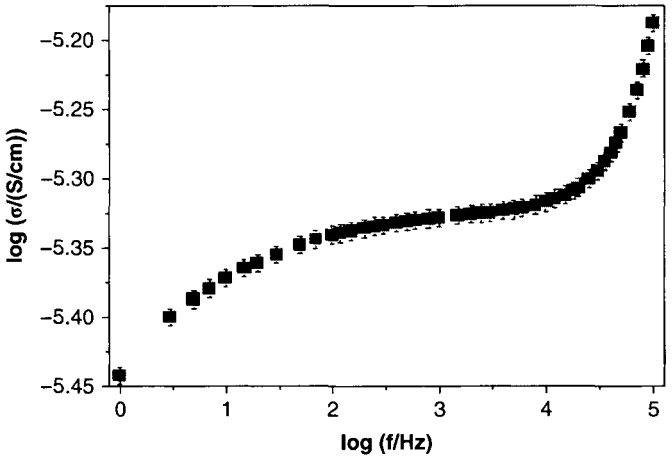

Figure 12.10 Plot of log σ(ω) vs. log f at 25°C. The textured specimen of Gum Arabica, Sample thickness = 0.1 cm, C.S.A. = 1 cm^2 at temperature 25°C [21].

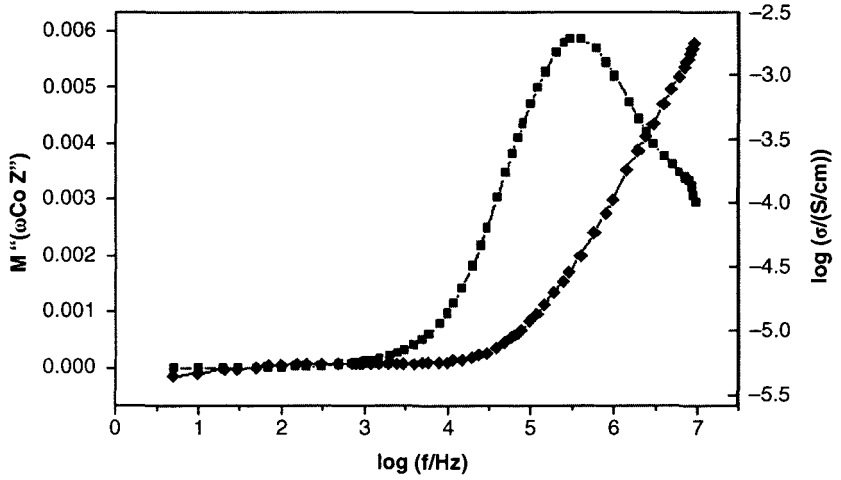

Figure 12.11 ■ Modular Spectrum, (left y-axis) ♦ Impedance Spectrum (right y-axis) of Gum Arabica specimen, frequency range 5 Hz and 9.5 MHz at 25°C (untextured specimen thickness = 0.1 cm; C.S.A. = 1.5 cm^2) [21].

Arrhenius plot: This plot provides direct information about ionic activation energy, phase transition, and electrical stability of nonelectronic conductors. Biomaterials are polymeric in nature and exhibit a certain Arrhenius nature. Hence, a review of the Arrhenius mechanism is needed. The plot of log σ vs. 1/T provides a straight line for ionic conduction and from the slope of the curve the sum of the activation energies. The Arrhenius behaviour of amorphous gum Arabica specimen was measured with a.c. at frequency 1 KHz between room temperature, 20°C–80°C, which is a thermally stable temperature range for gum Arabica biopolymer (as indicated by TGA study). The

activation energy E_a calculated [17, 20] from the slope of the Arrhenius curve was 0.17 eV which is comparable to other SPC and biopolymer like HUP [29]. The linear Arrhenius plot in the stable temperature range is given by,

$$\sigma = (\sigma_0/T)\, exp\, (-E_a / k_b T) \tag{12.13}$$

where σ_0 is the pre-exponential factor and E_a is the activation energy.

Figures 12.12a and 12.12b show [17, 20] in the Arrhenius plot a variation of d.c. conductivity between a temperature range 25–133°C. Table 13b shows the

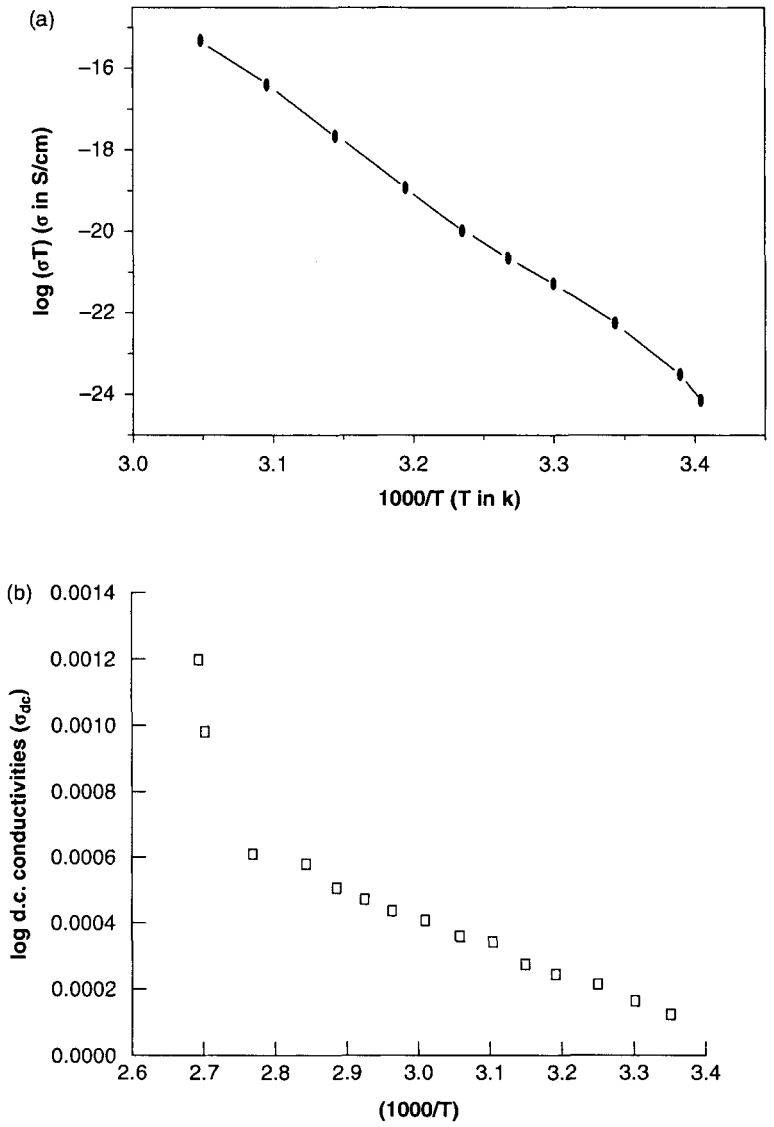

Figure 12.12 (a) Arrhenius plot, variation of a.c. conductivity of Gum Arabica between temperature range 25–130°C [17]. (b) Arrhenius plot, variation of d.c. conductivity of Gum Arabica between temperature range 25–130°C [17].

same for an a.c. experiment. It is clearly seen from the figure that the linear nature exists up to the stable temperature range (up to ~ 100°C). At a temperature above 100°C a nonlinear behaviour along with a peak in electrical conductivity at around 115°C is observed. Finally, the electrical conductivity tends to decrease above 115°C.

Volt-ampere characteristics: The study of volt-ampere (I-V) characteristics of a material is important with regard to electronic conduction in it. The nature of I-V characteristics provides information [30] electron and 'hole' contribution to the total electronic conductivity. In the following paragraphs, the d.c. I-V characteristics of gum Arabica will be discussed.

Figure 12.13 shows [17, 20] the volt-ampere characteristics of pure gum Arabica specimen at room temperature. Stabilized currents were recorded at various d.c. voltages. Following Wagner and Wagner [16], one can conclude that the observed stabilized current is due to electronic conductivity. The nature of the graph shown in Figure 12.13 can be explained by Wagner's explanation for the total electronic current and is given by,

$$I_T = I_- + I_+ \tag{12.14}$$

where I_- is the current due to electrons and I_+ is that due to holes. The experimental nature of saturation of the observed electronic current is also in accordance with Wagner's analysis [14]. Upon observation the result shows that the measured current tends to assume a saturation value with increasing potential difference, thereby showing that stabilized current is due to the electronic contribution (I_-).

However, in experimental studies it has been observed that gum Arabica specimen has a surface electrical conductivity which is very sensitive to local humidity. Moreover, it has been observed that the bulk electrical conductivity

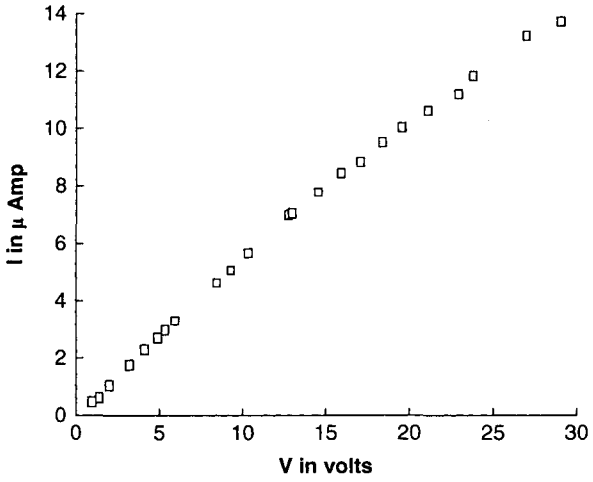

Figure 12.13 The volt–ampere characteristics of the Gum Arabica specimen at room temperature [17].

has little sensitivity to local humidity, saving it from prolonged exposure to the humid environment.

12.5 Electronic or Vibrational Properties

The physical appearance of pure gum and its complexes were often found to exhibit colour; therefore their optical absorption characteristics in the UV-VIS region were studied in order to investigate their optical absorption, absorbance region, and the photo-charge separation therein. The optical absorption in the UV-VIS region was measured for coloured complex of gum Arabica, and when and where it was important.

FTIR analysis: The study of biomolecules/biomaterials is an open field of research that has become a high priority in recent times. Investigation into the internal motion/dynamics of biomolecules/biomaterials is emerging as a new field of research geared towards: (i) An understanding of the physics of complex systems, (ii) an understanding of the internal mechanisms of a living system, and (iii) the development of clean and cost efficient technologies for use in various fields. Fourier transformed infrared spectroscopy for use as a powerful technique to extract molecular information such as energy difference (i.e. absorption of infrared frequency due to bond bending and bond stretching). The latter are the fingerprint of the functional group/ligand associated with a molecule.

An infrared (IR) spectrum only appears if the vibration produces a change in the permanent electric dipole of the molecule. The more polar a bond, the more intense the infrared spectrum arising from vibrations of that bond will be. Fourier transformed infrared (FTIR) spectroscopy for use as an emerging analytical tool capable of monitoring the IR spectrum of a biomolecule and hence the chemical information, with high signal-to-noise at high spatial resolutions. In an n-atom nonlinear molecule there are 3n-6 possible vibrational modes. The vibration includes bond bending and bond stretching. The energy difference between vibrational states corresponds to the energy level of infrared radiation (IR). Normally the two major regions in the IR spectrum of a molecule are the functional group region (7000 Cm^{-1} – 1500 Cm^{-1}) which includes the X-H stretching region and fingerprint region (1500 Cm^{-1} – 350 Cm^{-1}). The latter region is very important in biomolecular dynamics [31] and may provide much relevant information about the internal motion of the molecule and its related biomolecular function in living systems. The objectives of the FTIR spectroscopic [32] study are (i) to recognize the most reliable absorption frequencies for a particular functional group of a biomolecule, (ii) to use the group frequencies to distinguish the spectra of the sample, and (iii) to understand the factors which complicate (e.g. overtone and combinational bands, Fermi resonance, and most important for a biomolecule – hydrogen bonding) IR spectra and to be able to recognize the effect that these have on the spectra. In the work presented here, the vibrational characteristics of gum Arabica [33] along with its change in its polymerization effect are studied. Following will

be a brief discussion on the FTIR absorption spectra of prepared specimen of gum Arabica.

The gum Arabica powder specimen (S1) was collected from Merk (India) and was subjected to a sol-gel process along with pure water so that the polysaccharide host chain could form more complex higher polymers over those in normal powder form. Next, the sol specimens are extracted at initial state 30, 60, and 120 minutes. The experimental specimens (S2, S3, S4, and S5 respectively) were developed by adequate drying of the sols at environmental condition. The developed gum Arabica specimens (S2–S5) are supposed to exhibit a change in molecular structure over that in S1 due to prolongation of the sol gel process. FTIR analysis on pure gum Arabica was carried out to examine its molecular structure and dynamical information. The analysis was carried out at high resolution FTIR setup in a KBr window (shown in Figure 12.14).

Figure 12.14 shows the FTIR spectrum gum Arabica [33] specimen between wave number 350 – 4000 cm^{-1}. Broad peaks are obtained in the IR spectrum of gum Arabica at 3365.2 cm^{-1} (O-H stretching of carbohydrates), 2939.1 cm^{-1} (CH_2 asymmetric stretching), 1379.3cm^{-1} (CH, CH_2 and OH in-plane bending in carbohydrates), 1042.9 cm^{-1} (C-O stretching region as complex bands, resulting from C-O and C-O-C stretching vibrations), 7012.8 cm^{-1}, 641.7 cm^{-1}, and 603 cm^{-1} (pyranose rings). Figure 12.14a shows the distinctive absorption in the fingerprint region at 603, 641.7, and 7012.8 cm^{-1}. The three frequencies represent the characteristics of the bond stretching vibration of gum Arabica.

Figure 12.15 and Figure 12.16 show the comparison of the FTIR spectrum of the aforementioned gum Arabica specimens between wave number 600–607 cm^{-1} and 638–645 cm^{-1} respectively. Figure 12.15 shows that the characteristics vibration at 603cm^{-1} change while passing from S1 to S5, whereas Figure 12.16 shows the same corresponding vibration to absorption peak at 641.7 cm^{-1}.

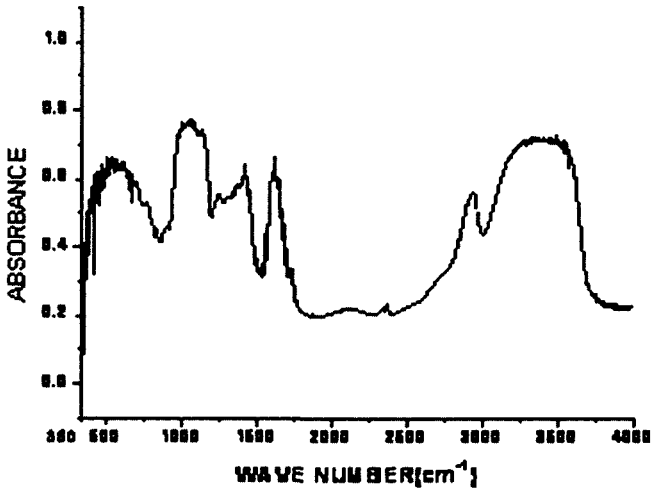

Figure 12.14 FTIR spectrum Gum Arabica specimen between wave number 350 to 4000 cm^{-1} [33].

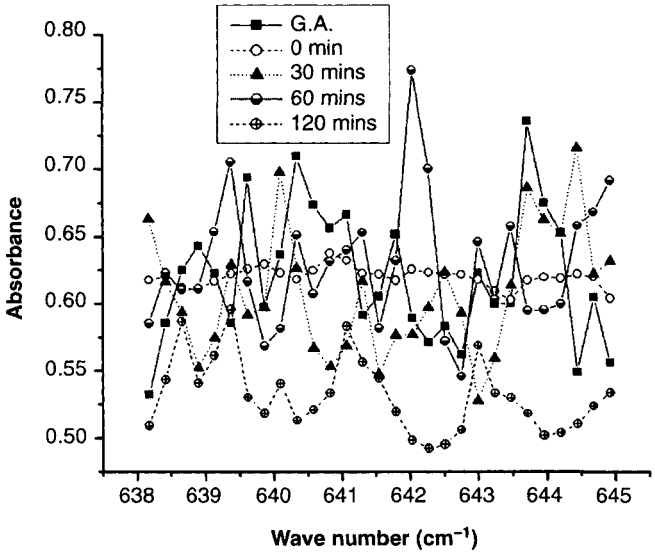

Figure 12.15 The comparison of the FTIR spectrum of different Gum Arabica specimens between wave number 638 to 645 cm^{-1} [33].

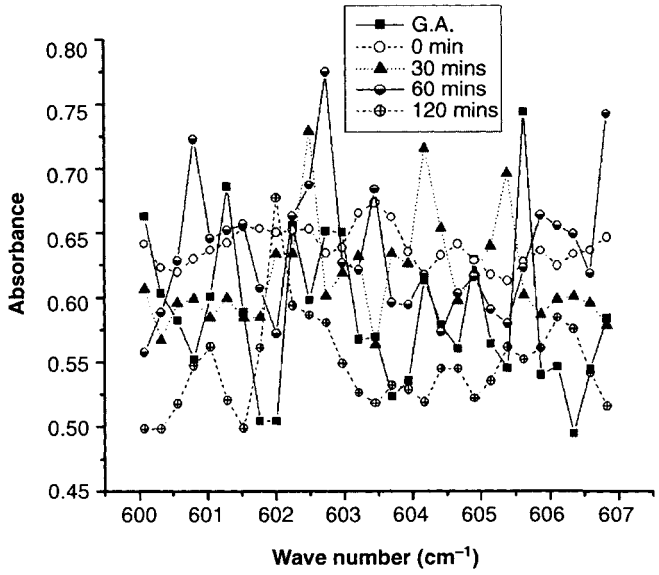

Figure 12.16 FTIR spectrum of different Gum Arabica specimens between wave number 600 to 607 cm^{-1} [33].

Table 12.1 summarizes the aforementioned change in absorption frequency at 7012.8 cm^{-1}. The overall results present a clear picture of change in the molecular structure in the biomolecule due to polymerization

A possible molecular level change in the gum Arabica structure was also recorded [33a] by a FTIR study. A deuterated specimen of gum Arabica was developed and the dried specimen subjected to a FTIR study. Figure 12.17

Table 12.1 Comparison of the FTIR spectrum peak for S1 – S5 around wave number 704.8 cm^{-1}.

Wave Number	G.A. (Absorbance)	0 Min (Absorbance)	30 Mins (Absorbance)	60 Mins (Absorbance)	120 Mins (Absorbance)
704.47749	0.59586	0.5822	0.66863	0.59402	0.50622
704.71858	0.6251	0.58692	0.65667	0.64203	0.51766
704.95967	0.61138	0.58325	0.72432	0.66985	0.51106
705.20077	0.81885	0.58042	0.66517	0.64703	0.50187
705.44186	0.59711	0.58188	0.59367	0.75077	0.4769

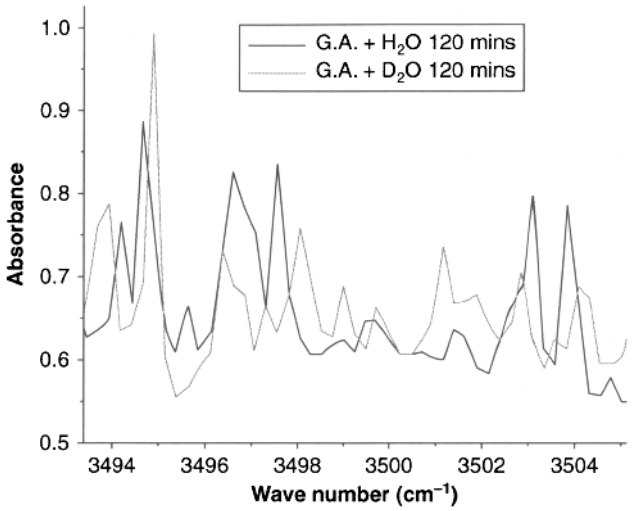

Figure 12.17 FTIR absorbance spectra of Gum Arabica developed with D_2O treatment compared with that of same treated with H_2O [33a].

shows the result of D_2O treatment, and also compares it with a normal H_2O treated specimen of gum Arabica. The overall results give a clear picture for change in molecular structure in the biomolecule due to D_2O treatment.

12.6 Enhancement of Electroactivity

Electroactivity in a polymeric system can be tailored according to the requirements of the application. A brief account of the tailoring of electroactivity of gum Arabica is presented in this section. In an earlier work [34], a solid specimen of gum Arabica complex was prepared by forming complexes with organic citric acid at different concentrations. The resulting specimens were used to investigate electroactive properties and their enhancement with an increase in acid concentrations in it. In the study the developed complex specimens were found to behave like an admixture of PE and ICP by their mixed conducting nature. When the gum Arabica was blended with pure citric acid to form a complex, the dissociated ions from the acid were free to take part in the transport process in the solid polysaccharide matrix under an external electric field so that it could be compared to ICP. The solid specimens for electrical measurement were developed by adequate drying at an environmental condition. Figure 12.18 shows that the slope of the linear portion in each graph increases with increasing acid concentration in the specimen. The variation of d.c. conductivity with added acid concentration was recorded and showed that conductivity increases with the increase in acid concentration nonlinearly up to a moderate concentration; however, it showed a linear variation in the high concentration region of the measured range of concentration. The increase in electronic conductivity with acid concentration is definitely an interesting aspect of this complex of EABP. The developed acid

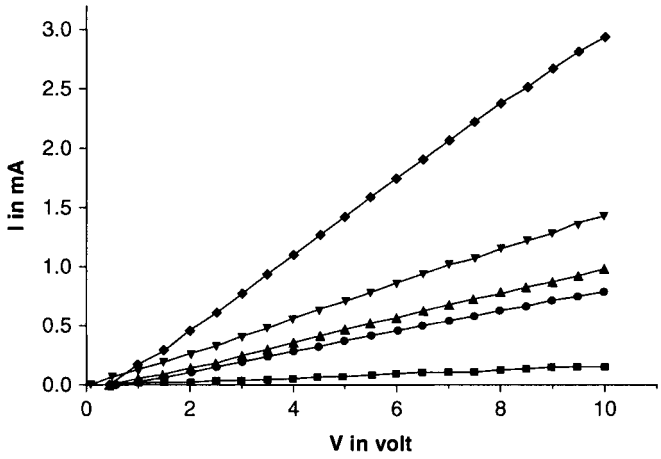

Figure 12.18 V–I characteristics of Citric acid complex of Arabica (specimen size A=1 cm^2, d = 0.3 mm) [34].

complex was found to exhibit an Arrhenius nature. The electrothermal activation energy for the specimen was estimated in three distinct regions of the composites. The estimated values of the activations energies were found to be 0.31 eV, 0.13 eV, and 0.003 eV at citric acid concentrations 3%, 5%, and 10% by weight respectively in the gum Arabica complex. The work confirms that its electroactivity can be tailored and controlled by complex formation with organic compound. The aforementioned work provides a detailed account of the tailoring of electroactivity of gum Arabica with the use of citric acid in an all green way. This highly potential complex of gum Arabica can be used in interdisciplinary research and technology.

Formation of complex at the molecular level may be analyzed from the study of IR absorption spectra. It provides information about energy difference between vibrational states, originating from bond bending and bond stretching. Figure 12.19 shows the FTIR transmittance spectrum of the citric acid complex of gum Arabica and it also compares it to that of the pure gum Arabica. The functional group region appears from 4000 cm^{-1} to about 1550 cm^{-1}, reflecting mostly the characteristics bond stretching nature in FTIR absorption spectrum of pure gum Arabica.

Due to the addition of citric acid, the acid radical replaces OH group of the polysaccharide and performs the plasticizer effect which is responsible for the enhancement of the ionic conductivity of the matrix. The free H^+ ion plays a dominant role and, in fact, the dissociation of the weak acid leads to high conductivity. Thus, the resulting complex was a mixed conductor of ion and electron. This observed phenomenon is apparently like a phase transition, from ion conducting phase to ICP phase. The observed nature of optical absorption (shown in Figure 12.19a) provides clear evidence of the formation of complex molecules of polysaccharide unit with citric acid. The decrease in absorption frequency in the spectrum was due to the substitution of OH group by a heavier radical group of citric acid. The results of FTIR study in Figure 12.19

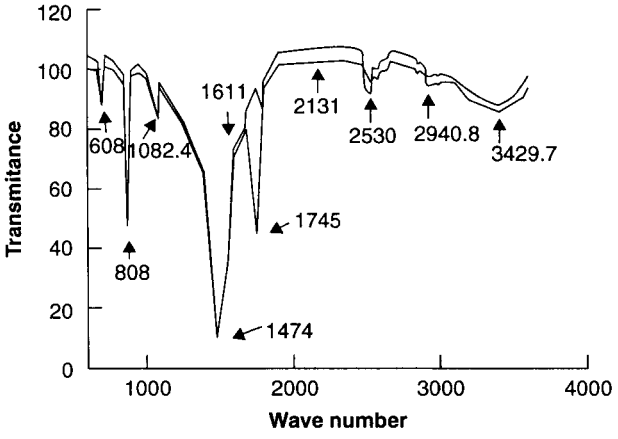

Figure 12.19 FTIR transmittance spectra of Citric acid complex of Gum Arabica (upper graph) compared with that of pure Gum Arabica (lower graph) [34].

provide interesting information about the molecules in gum Arabica. The region 1550 cm^{-1} – 625 cm^{-1} is a fingerprint region showing a large number of peaks from the molecules. The peak that is seen around 1745 cm^{-1} in the FTIR spectrum of the citric complex of gum Arabica, is the characteristics of the –COOH group from citric acid. The existence of acid unit in gum Arabica is responsible for its electrical conduction. Moreover, carbon double bond and triple bond also provides parallel support to the conduction mechanism in its bulk structure. The existence of unsaturated internal/side unit leaves a good scope for its complex formation as needed in the work.

12.7 Application Potential in Material Science

12.7.1 Gum Arabica and Its Scope of Application

12.7.1.1 Complex of Gum Arabica

The overall nature of the electrical conduction of the biomaterial gum Arabica as discussed in section 12.4.1 is like that of synthetic polymer doped with inorganic salts. The electrical behavior of gum Arabica has encouraged the undertaking of a study on its salt complexes. Following are some of the possible various salt complexes of gum Arabica that have been developed in a chemical sol-gel process and studied experimentally. The different inorganic salts/compound used in this purpose is $CuSO_4$, LiOH, H_3PO_4, etc. The results of electrical studies on these complexes showed enhancement of electrical conductivity over the pure material by a moderate degree. The chemical stability of the complexes was also found to be good. However, the degree of enhancement of electrical conductivity was not high enough (except H_3PO_4). Further study on H_3PO_4 complex of gum Arabica showed that it forms ionic hydrogel at specified conditions. The same phenomenon was also observed

with ammonium di-chromate and ammonium hepta-molybdate salt. Ionic gels, their characteristics, and their study are very interesting and have many potential applications. In the following subsections, the details of preparation technique, characterization, and the possibility of electrochemical application of the mentioned complexes are discussed [35].

12.7.1.2 Hydrogels

Hydrogels are polymer networks which swell in water without dissolving. They are usually made of hydrophilic polymers crosslinked by various interactions in chemical bonds such as hydrogen bonding, ionic interactions, and hydrophobic interactions. Hydrogels containing a significant portion of hydrophobic polymers can also be made. The properties of hydrogels can be controlled by the careful choice of the type of monomers [36]. Hydrogels have a high water capture capacity important for applications in biological environments. Hydrogels possess good biocompatibility due to their unique properties such as low interfacial tension with surrounding biological fluids and their rubbery nature, which can minimize mechanical and frictional irritation. They can be designed to respond (i.e. either swell or shrink) to changes in environmental conditions such as pH, temperature, electrical field, ionic strength, salt type, solvent, and light. It is these unique properties that have made hydrogels useful in various applications.

Hydrogels can be classified into matrix, film (or membrane), and microsphere according to their physical appearance; synthetic hydrogels and natural hydrogels according to their origin; homopolymer, copolymer, block copolymer, interpenetrating polymer networks (IPN) and semi-IPN hydrogels based on their structure; and physical gels and chemical gels based on their nature of cross-linking.

Chemical gels are formed by the cross-linking of polymers by chemical reactions whereas physical hydrogels are formed by physical interactions between polymer chains. These interactions include hydrogen bonding, hydrophobic interactions, and ionic interactions. There are several types of physical gels present, within which some hydrogels are made of polyelectrolyte complexes. Ionic interactions between two oppositely charged polyelectrolytes lead to the formation of polyelectrolyte complexes, which swell in water. Hydrogels have been extensively used as drug delivery systems. Since hydrogels are hydrophilic in nature, they are generally very much biocompatible. They are also used as biomaterials and possess several applicabilities in biotechnology, agriculture, etc.

12.7.2 Biopolymer Gel

High ion/proton conductive solids are highly important in various electrochemical applications such as electric cells, sensors, etc. The water-based materials face several disadvantages [37]. Gels prepared by the sol-gel route are materials whose macroscopic appearance is solid-like but exhibit liquid-like phenomenon microscopically. This is due to the presence of large numbers

of water-filled micropores and mesopores in gels. Proton conducting gel conducts electricity due to the transport of H^+ and OH^- ions in it. The characteristics and applications of many ion/proton conducting gels have already been reported on [38, 39]. In the following section an experimental study of the different electrochemical aspects of a proton conductive ionic gel of gum Arabica [35] will be presented.

12.7.2.1 Development of Gum Arabica Gel

Orthophosphoric acid was added to aqueous solutions of gum Arabica in different concentrations by weight percentage and stirred for uniform mixing. Resulting solutions were then treated by sol-gel like process. The developed solutions were then charged in a special protocell of Area 2 cm^2 and thickness around 0.5 mm between two finely polished non-attackable electrodes for experiments. It was found that H_3PO_4 (conc.) 16% by weight of gum Arabica produces gel electrolyte on slow cooling and reaches equilibrium condition after 48 hours. The electrolyte could not maintain its gel nature in pellet form due to the increase in surface to volume ratio as gel collapsing occurs. So the gel electrolyte was put in a special glass cell of required geometry to decrease the surface to volume ratio. In a similar fashion gels of chromium and molybdenum metallic ligand were prepared using a solution of ammonium dichromate and ammonium heptamolybdate (8% by weight). During a prolong sol-gel process at a temperature of about 100°C, the said ammonium compounds were thermally decomposed to leave metallic oxides which act as a crosslinking agent to the hydrated polymeric chain of gum Arabica. The time required to attain an equilibrium condition for these two gels was less than H_3PO_4, one hour for the molybdenum gel.

The formation of the above mentioned polymeric gels follows few possible mechanisms [40, 41]. Dissociated phosphate, di-chromate, and molybdate ions form chemical bonds with the monomer units of gum Arabica polymer. The resulting charged monomer units named polyelectrolytes were then cross-linked into an integrated spatial network in water solvent. The repulsion between non-neighboring monomer ion units possibly followed the Coulomb interaction over the van der Waal interaction (for strongly charged polyelectrolytes) that favours contact with water solvent by minimizing polymer-polymer contacts. Also, the counterions being produced simultaneously with the charged monomer units do not move out into the surrounding solution even though they are free. To ensure electroneutrality of the gel these counterions remain within the network and exert osmotic pressure. The combined effect of the repelling force of polyelectrolytes and the osmotic pressure of counterions are responsible for polyelectrolyte gel swelling in water. Increase in the concentration of mobile counterions inside the gel corresponds to the increase in osmotic pressure. Gels thus developed were found to be thermo-irreversible. On the other hand, when polyelectrolytes attract each other the gel state collapses, not favoring polymer-solvent contacts. If the counterions remain bound with the chain, they form an ion pair.

Ion pairs attract each other due to dipole-dipole interactions that form multiplets, which act as additional physical crosslinks. This leads to the formation of a new super-collapsed state of the gel. The super-collapsed state corresponds to the state of practically dry gel. The developed gels were found to satisfy requirements as an electrolyte for practical implications of a cell [42], which are: (i) They are a good conductor of ions and insulator of electrons (holes). Electronic conductivity should be five orders of magnitude lower than the ionic conductivity ($\sigma_{el} / \sigma_{ion} = 10^{-5}$), (ii) they possess good interfacial contacts with electrode material, (iii) they are a cost effective and readily accessible raw materials resource and, (iv) they have electrical conductivity $\geq 10^{-4}$ S/cm. The outcome of the chemical composition of orthophosphoric acid or cross-linking agent with gum Arabica biopolymer meets the above mentioned criteria. The ionic conductivity of the composition increases over that of pure gum Arabica as H_3PO_4 dissociates into its constituent ions. In this reported study, a proton conductive gel was developed with gum Arabica and H_3PO_4 by means of the sol-gel route, which was found to be a good member for various electrochemical applications (e.g. cell). Gels were also prepared by incorporating a metallic ligand of chromium and molybdenum compound as a cross-linking agent with gum Arabica biopolymer. Following, some experimental results on gum Arabica gel are analyzed.

The XRD pattern of H_3PO_4 gel shown in Figure 12.20a provides an interesting feature of intensity distribution. The peak of the distribution is shifted considerably in comparison with pure gum Arabica. XRD pattern indicates that the gel is sensitive on humidity. As it changes from a gel state to a collapsed nearly dry state, the increase in RDF reveals the long range ordering of molecular distribution which is indicated by the flattening of the curve and change in peak pattern.

The DSC results given in Figure 12.20b show no interim phase transition up to a temperature of 80°C, and the water content within its pores remains practically unaltered. This is also an indication of the stability of this gel in an electrochemical application up to 80°C.

The SEM photograph of a gel surface shown in Figure 12.21 shows no new phase transformations except from gel formation of the complex. The pictures clearly indicate the microporous structure of the ion conducting gel.

Figure 12.22 shows the variation of a.c. conductivity with an increasing concentration of H_3PO_4 complex of gum Arabica at 25°C. The observed variation may be described by Jonscher's power law. Increase in phosphate ions and mobile counterions enhance the bulk conductivity of the specimens, which is summarized in Table 12.2.

Table 12.2 Conductivity of H_3PO_4 complex of G.A with increasing H_3PO_4 concentration.

G.A + x % H_3PO_4	x = 1	x = 4	x = 7	x = 10	x = 13
Conductivity (S/cm)	$3 * 10^{-6}$	$5 * 10^{-6}$	$1 * 10^{-5}$	$3 * 10^{-5}$	$1.3 * 10^{-4}$

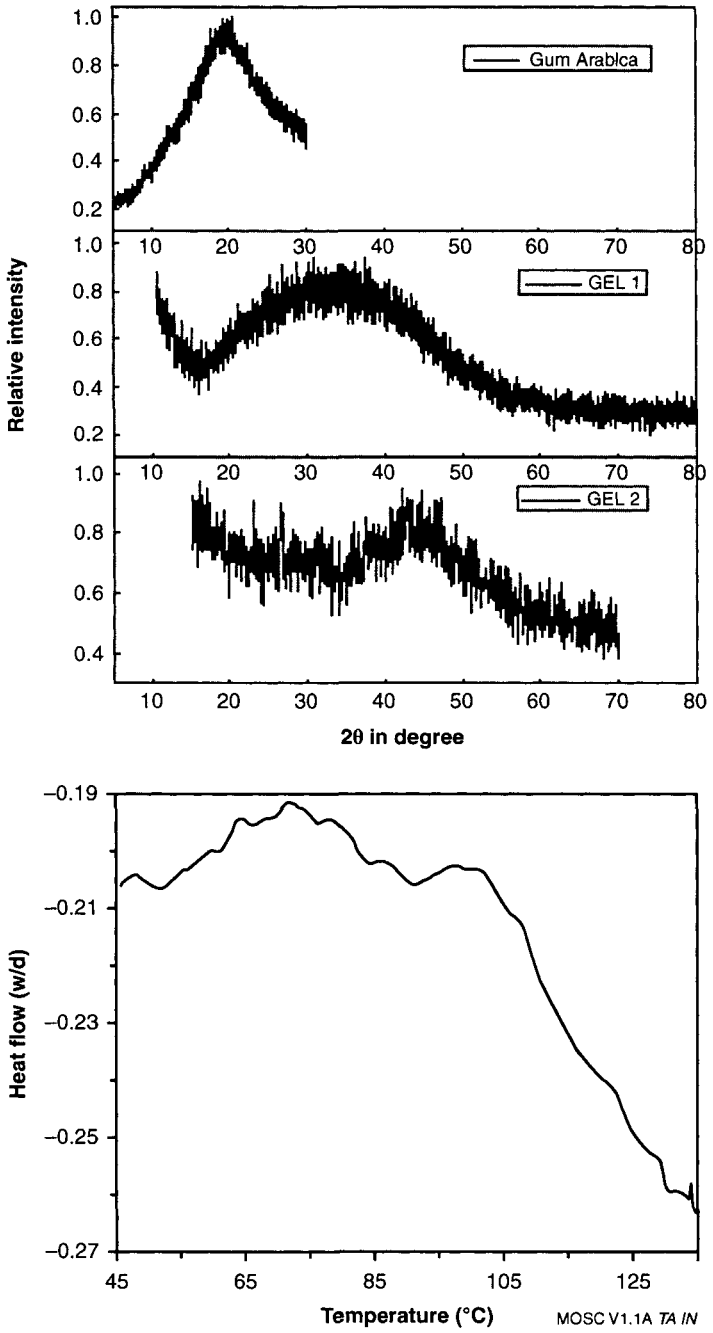

Figure 12.20 (a) Relative Intensity vs 2θ in degree for pure Gum Arabica, Gel 1& Gel 2 correspond to H_3PO_4 Gel; Gel 1: Gel in Liquid form; Gel 2: Gel in lower water content than the normal state [35]. (b) Result of DSC measurement on Gum Arabica Gel [35].

The electrical conductivity of H_3PO_4 – gum Arabica gel electrolyte was estimated to be $\sigma \sim 1.8 \times 10^{-3}$ S/cm. The enhancement of conductivity in a gel state is more prominent than the liquid specimen, which ensures that the gel state has a higher conductivity over its liquid state. The conductivity of the

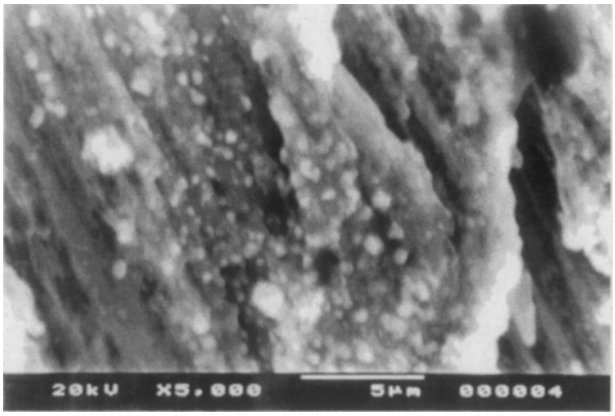

Figure 12.21 SEM picture Gum Arabica gel surface at Magnification 5000 [35].

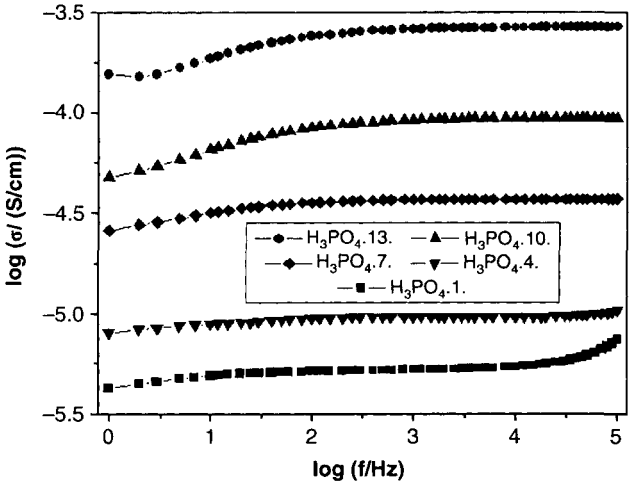

Figure 12.22 log σ vs log f of H_3PO_4 complex of Gum Arabica [35].

chromium and molybdenum gel of gum Arabica was thus estimated to be of the order of 10^{-2} S/cm. The respective conductivity plot with frequency is shown in Figure 12.23.

The observed I-V characteristics for the developed gels are shown in Figure 12.24. The linear nature was found for H_3PO_4 gel complex. The other two gel complexes exhibit nonlinear I-V characteristics, which are probably due to the formation of ion channels by the d-band metallic ligands of sub-micron dimensions. The observed characteristics may be due to formation of ion channels within the bulk gel. The same characteristic has been observed for natural gels [43].

Figure 12.25 shows a near straight line Arrhenius behaviour of the H_3PO_4 gel at 1 KHz with decreasing activation energy $E_a \sim 0.088$ eV over that of pure Gum Arabica which 0.17 eV. The DSC result indicates its stability up to 80°C, the temperature variation of total conductivity is observed between room temperature and 80°C.

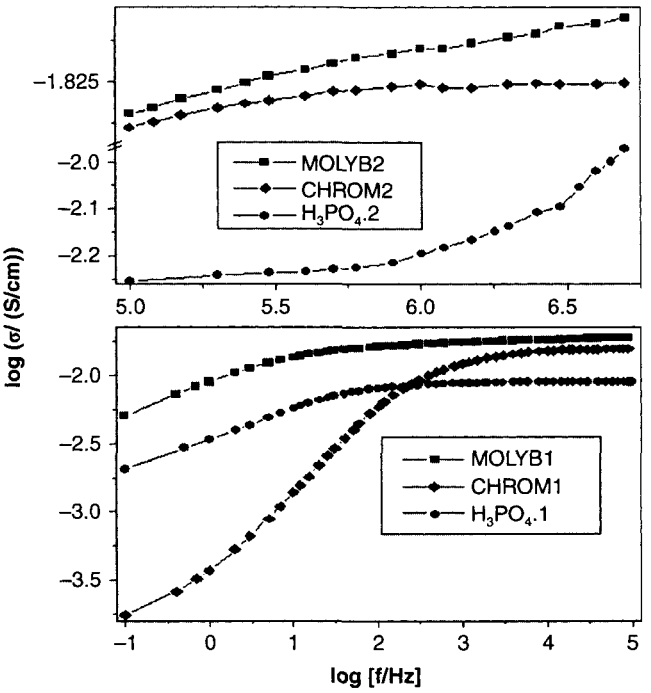

Figure 12.23 log σ vs log f of H_3PO_4, Chromium and Molybdenum Gel of Gum Arabica at 25°C. Frequency Range of the lower picture is 0.1 Hz – 100 KHz and 100 KHz – 5 MHz for upper picture [35].

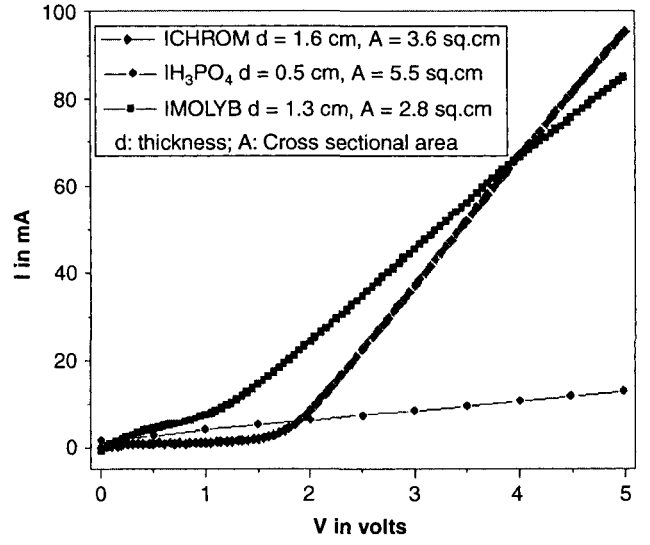

Figure 12.24 I-V characteristics of H_3PO_4, Chromium and Molybdenum Gel of Gum Arabica [35].

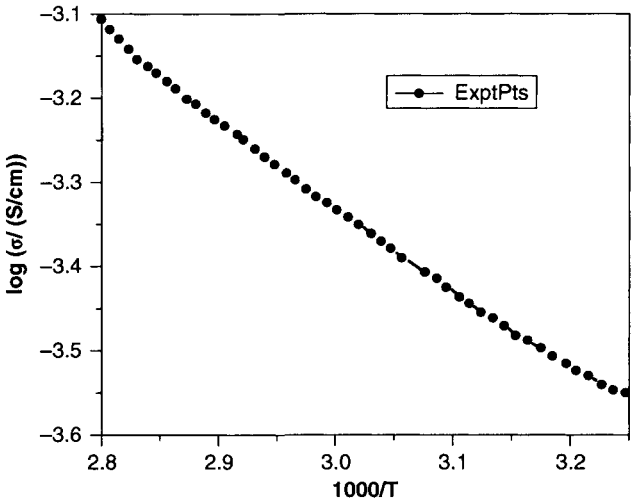

Figure 12.25 Arrhenius plot of H_3PO_4 Gel of Gum Arabica [35].

The ionic gel of H_3PO_4 thus developed was utilized in developing an electrochemical cell with non-attackable electrode pair. The cell exhibit an e.m.f of 1.1 Volt with moderately high current density, the overall results are encouraging, The gels are developed using biopolymeric host Gum Arabica and inorganic compounds H_3PO_4, Ammonium di-chromate and Ammonium heptamolybdate were found to be ionic in nature with a higher electrical conductivity over their corresponding sol state. Moreover, they have very good thermal and electrochemical stability for electrochemical applications. I-V characteristics for the chromium and molybdenum compound based biopolymeric gels show a marked difference compared to that of H_3PO_4 gel.

12.7.3 Nanocomposites

There is a need for the study of nanosized particles, composites, and devices in order to find ways to build powerful materials towards application in the fields of electronics, physical science, material science, biological science, and pharmacology. Nanocomposites (NC) occupy a leading role in this field. Organic-inorganic nanocomposites [44] are of considerable interest and classified into two types: nanoparticles (NP) and nanostructured layered materials. In a recent work the author and his co-workers investigated the material potential of developed nanoclusters and organic nanocomposites. The 3d transition metal oxides display a broad variety of electronic, magnetic, and structural material properties. Electrical transport through low dimensional nanocomplex has developed considerable interest in utilizing their intrinsic properties to exploit a possible role in new types of nanoscale devices [45, 46]. Synthesis of nanostuctures by natural self-assembly of different nanoclusters in the dielectric substrate of gum Arabica biopolymer is employed [44]. These clusters occupy some localized position in the background medium; providing

3-D quantum confinement and additional localized energy levels for carriers, which causes a change in the electrical characteristics of nanocomposites.

12.7.4 Metallic Sulphide Nanocomplex of Gum Arabica

The present discussion [47] deals with the formation of natural self-assembly of CuS nanoclusters in dielectric substrates of gum Arabica biopolymer. Low concentration of ammonium complex of copper oxide solution was dissolved in gum Acacia Arabica solution at 60°C and stirred. H_2S gas was passed in the same environment for 1 minute. Heating the resulting solution to about 100°C evaporates any possible trace of ammonia. The resulting nanocomplex was caste in the form of very thin film by spin coating technique. The developed specimen was used for experimental investigations, namely TEM, XRD, and electrical experiments such as impedance spectroscopy, and the Arrhenius plot and I-V characteristics were measured in the applied field direction perpendicular to the 2-D plane.

Microstructural information was obtained by a transmission electron microscope at two different magnifications, and the results in Figure 12.26a and Figure 12.26b show spontaneous nanacluster formation in the base matrix. The maximum particle size was estimated as 9.5 nm.

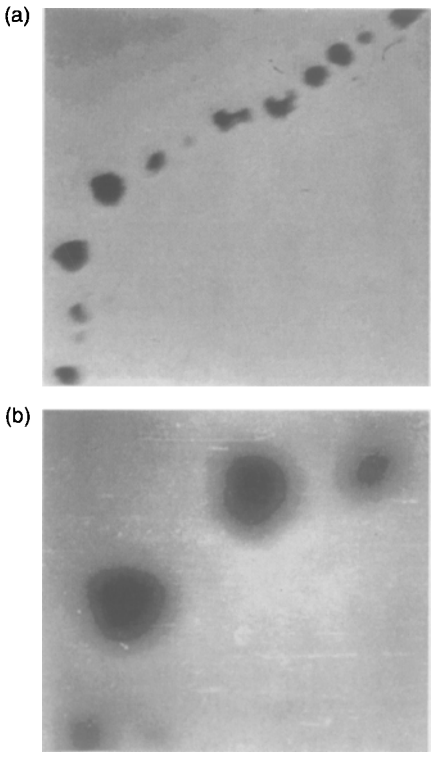

Figure 12.26 (a) TEM picture of CuS nano complex of Gum Arabica at magnification 100K and (b) 250 K [10]. (b) TEM picture of CuS nano complex of Gum Arabica at magnification 250 K [10].

X-ray diffraction was obtained by Cu K_α radiation shown in Figure 12.27. The background of the pattern in Figure 12.27 indicates the amorphous nature of the gum Arabica matrix. The superimposed peak over the background indicates the formation of nanocluster of estimated sizes comparable to that of TEM study.

The result of impedance measurements of the bulk specimen of gum Arabica CuS nanacomposite between two copper electrodes carried out between a frequency range 1 Hz–100 KHz is shown in the Cole-Cole plot in Figure 12.28.

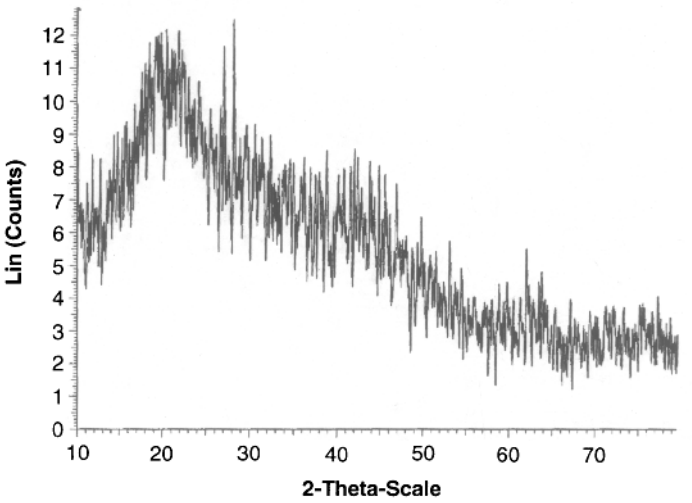

Figure 12.27 X-ray diffraction of CuS dispersed Gum Arabica matrix (powdered) [10].

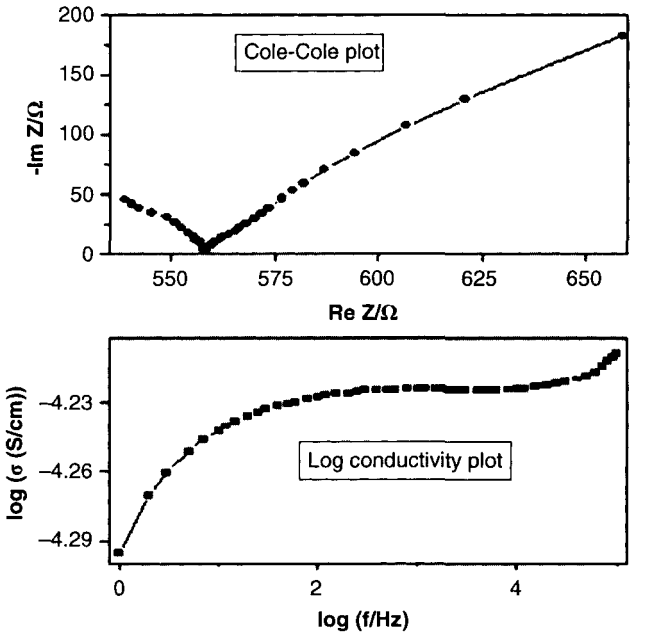

Figure 12.28 Cole-Cole plot and Log conductivity plot of bulk sample (d = 0.6 mm, C.S.A = 1.8 cm^2, p.d. = 1V) [10].

Temperature variation of impedance at 1 KHz was recorded by the same impedance analyzer. The pattern shows near Arrhenius-type behaviour and the overall conducting nature is ionic.

Figure 12.29 shows the I-V characteristics of thin specimen at room temperature, and 253 K were observed by high current source measure unit and plotted by using Metrics Interactive Characterization Software. The overall nature of I-V characteristics is an apparent indicator of the formation of localized energy levels in the host background. The formation of localized energy level may be attributed due to 3-D confinement of quantum dot-like ionic nanoclusters within the dielectric substrate.

Important observations made from the study of nanocomposites: The chemical route for synthesizing nanocomplex in a biopolymeric host matrix has been successfully implemented. It has been found [44] that in the developed nanocomposite with gum Arabica as host background, the capping of the nanoclusters become complete and highly stable compared to other capping agents [e.g. polyvinyl alcohol (PVA)]. In the study [44], nanoclusters of CuS, ZnS, and Sulphur in the gum Arabica background were investigated and the overall results were found to be very interesting. The work put special emphasis on the electrical characterization of the developed specimen along with some standard techniques. Earlier studies [48, 49] showed that 0-dimensional and 1-dimensional quantum system exhibited interesting features of electrical transport through them. Following is a brief account of the investigation and and a summation of subsequent studies where oxide (M_xO_y: M- metal atom) nanoclusters were developed in gum Arabica background. The surfactant action of gum Arabica is found to be better than that of PVA. The developed nanocomposites were found to exhibit a mixed conducting behaviour. Because

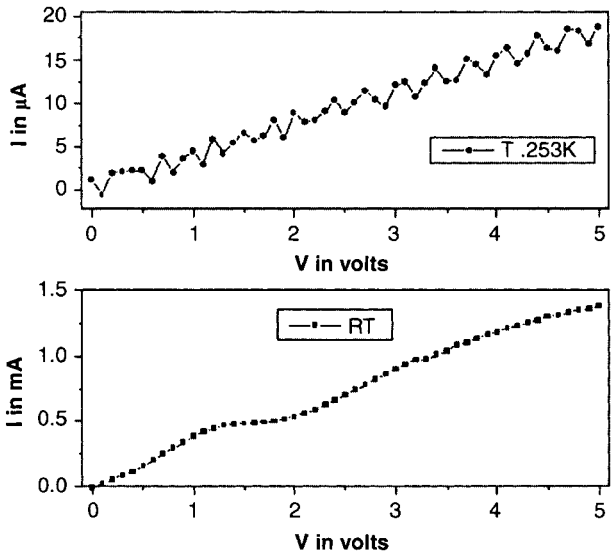

Figure 12.29 I – V characteristics of thin specimen at room temperature and 253 K [10].

of quantum size effects or confinement [48], the electronic motion becomes quasi-one-dimensional, and changing the Fermi energy of a quantum system leads to oscillations in the density of states, the thermal scattering rate, and the conductivity. The quantum size effect in quasi-one-dimensional electrical conduction has been reported [49]. In an earlier investigation, simple plots (shown in Figure 12.30) of conductance G vs. back-gate voltage V without the tip present show complex patterns of Coulomb blockade peaks with uneven spacing and height. All the above mentioned features were observed at low temperature. It is apparent that this mentioned study indicates a manifestation of quantum phenomenon at RT. An AFM picture of the developed ZnS nano-cluster in gum Arabica background is shown in Figure 12.31.

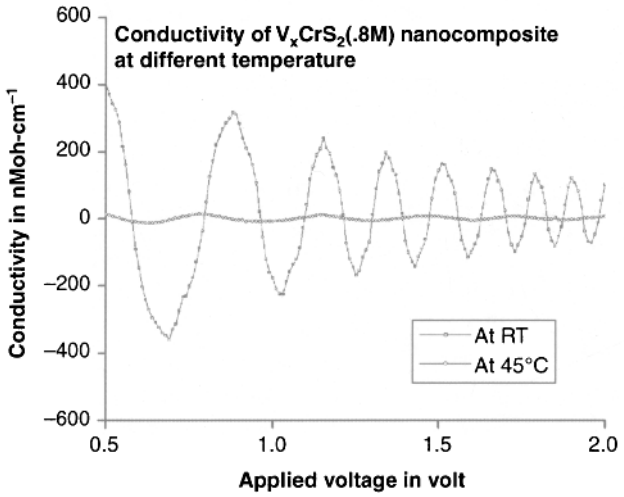

Figure 12.30 Comparison of σ-V variation for the developed nano-complex specimens in Gum Arabica matrix measured at RT and at 45°C with voltage step 10 mV. Sample thickness 0.096 cm, CSA = 1.62 sq. cm for RT and 0.112 cm, CSA = 1.2 cm for 45°C [63a].

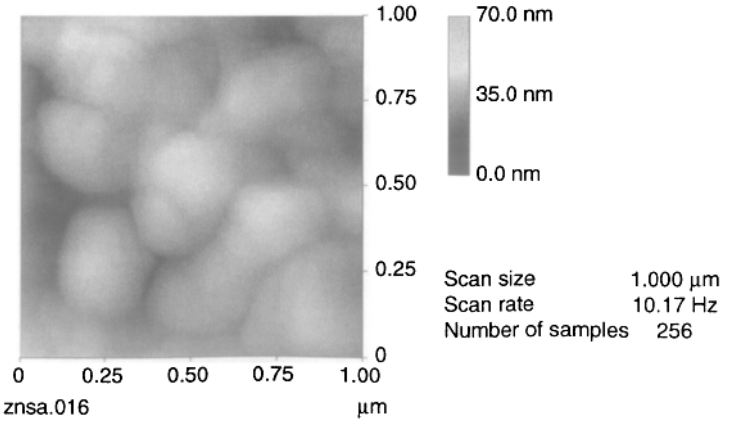

Figure 12.31 AFM picture of ZnS Nanocomplex specimen of Gum Arabica [63b].

12.7.5 Development of Carbon Nanoparticle

In this subsection, the development of carbon nanoparticle (CNP) in gum Arabica background is highlighted. Figure 12.32 shows the XRD pattern of the NC specimen and compares it with that of the pure gum Arabica specimen. The XRD pattern shows the amorphous nature of the NC material, however, it is clear that due to dispersion of CNP cluster the peak slightly shifted towards higher 2θ value over the background of the pure material. The obtained pattern definitely ruled out superlattice formation, however, the calculated coherence length from the Scherrer equation indicates the in growing anisotropy over pure background material due to the presence of CNP clusters in the composite.

Figure 12.33 shows an AFM view of NC specimen with low CNP concentration (with 6hr passage of C_2H_2 gas). The picture provides a probable confirmation of formation CNP cluster in background gum Arabica. The CNP cluster size estimated from the picture was about 18–19 nm.

The result of impedance spectroscopy for NC specimen (18hr passage of C_2H_2 gas) measured at room temperature 20°C is shown in a Cole-Cole plot in Figure 12.34. The measured conductivity dispersion also shows an initial increase of conductivity with increasing CNP concentration. The bulk conductivity estimated from data is 4×10^{-5} S/cm, which is 25 times higher than that of pure background material.

Figure 12.35 shows the Arrhenius plot for the specimens for different CNP concentrations. It shows a near straight nature in all the cases with decreasing activation energy (Ea) with an increase in CNP concentration. However, at higher CNP concentration Ea increases again and was estimated to be 0.214 eV at 18hr passage of C_2H_2 gas, which is more than that of pure gum Arabica.

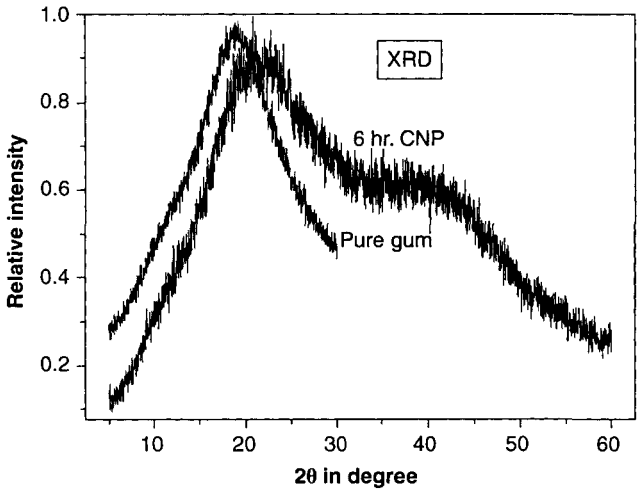

Figure 12.32 XRD pattern of the NC specimen and compares with that of pure Gum Arabica [10].

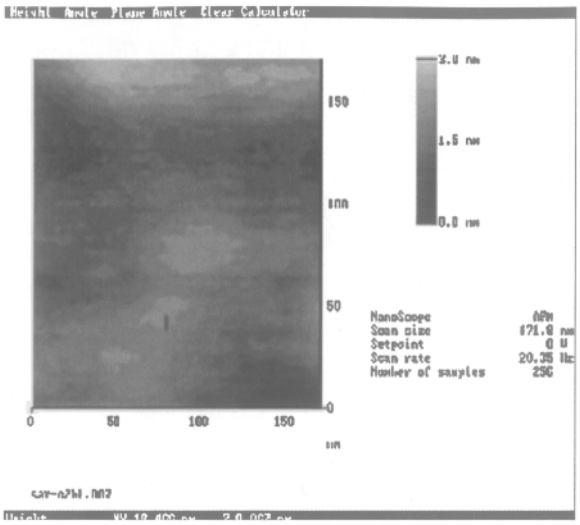

Figure 12.33 AFM view of NC specimen with low CNP concentration [10].

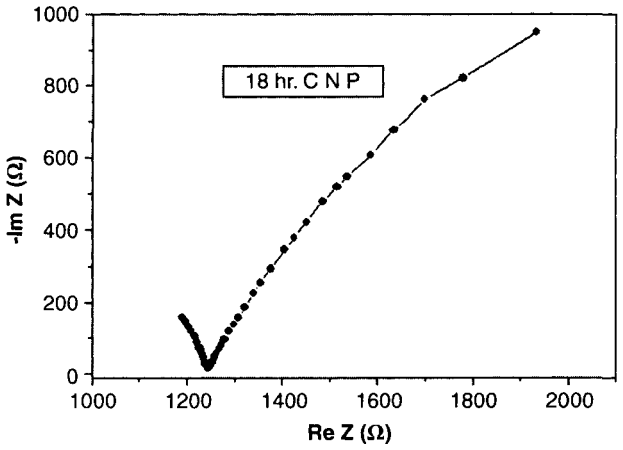

Figure 12.34 Cole-Cole plot of CNP Nanocomplex specimen of Gum Arabica [10].

Figure 12.36 shows the variation of a.c. conductivity with frequencies for samples with various CNP concentrations. It shows that specimens may accommodate more CNP and conductivity increases with increase in CNP concentration. The observed conductivity spectrum shows that plateau region increases with an increase of CNP concentration. This very feature is an indicator of the increase of d.c. conductivity with increasing CNP concentration as shown in Table 12.3

The results of ion transference number measurement summarized in Table 12.3, shows the variation of ionic and electronic conductance of the NC's with increasing CNP concentration. The results show an increase in electronic conductivity with CNP concentration.

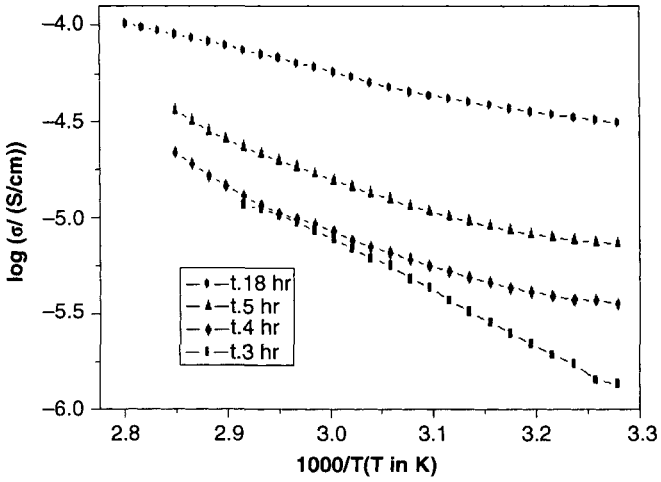

Figure 12.35 Arrhenius plot for variation in CNP Nanoparticle concentration [10].

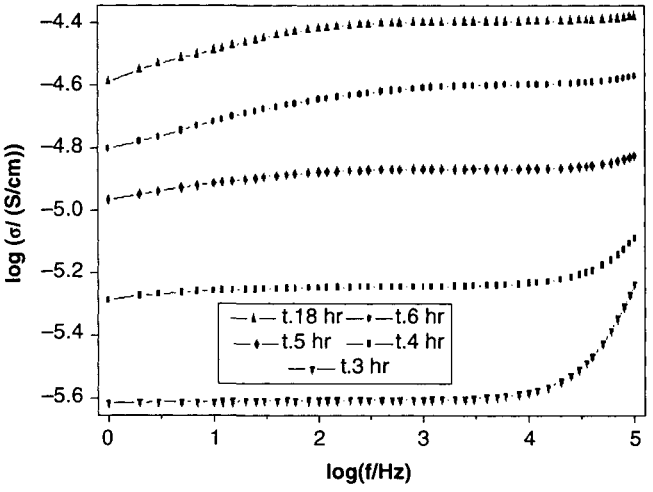

Figure 12.36 A.C. Conductivity variations with CNP concentration [10].

In the above mentioned work, the dispersion of CNP leading to a NC is purely due to 'natural self-assembly', which provides NC in an amorphous state. The average size of CNP clusters is in quantum dot regime and thereby provides additional electron energy states for carriers leading to size effect which enhanced the electronic conductivity with increasing CNP concentration. Due to the presence of CNP clusters of a size that was comparable, or even more than that of the average grain size of pure background material, the former causes microscopic distortion, which in turn enhances the ionic conductivity of background material. These two mentioned mechanisms facilitate the NC to be a well-mixed conductor with the required stability.

The nature of electrical conduction in the developed specimen of the mentioned nanocomposites exhibits their distinct character with the manifestation

Table 12.3 Comparison of electro-actvity of carbon nano particle complex of Gum Arabica.

Specimen	τ_{ion}	$\tau_{electron}$
Pure Gum Arabica (GA)	0.99	0.01
GA + 3 hr deposition of CNP	0.85	0.15
GA + 4 hr deposition of CNP	0.81	0.19
GA + 5 hr deposition of CNP	0.78	0.22
GA + 6 hr deposition of CNP	0.74	0.26
GA + 18 hr deposition of CNP	0.60	0.40

of finite discontinuities in their I-V characteristics. In fact, the features mentioned are observed in all the different nanocomplexes that are studied. The jumps or magnitudes of discontinuities are found to have temperature dependence. The apparent noise-like curve for I-V characteristics may be attributed to the statistical fluctuations. The said fluctuations are reproducible and may be analyzed from the statistical physics since the very existence of localized energy levels of nanoclusters and thermal energy of the carriers are competitive over the range of temperature in which the experiments are carried out. In conclusion, the said discontinuities in I-V curve are the characteristics of nanocomposite and a detailed study of it may be a method of characterization of nanocomposite.

12.7.6 Photosensitive Complex

In an earlier attempt [50], complex materials from biological ingredient, namely fine chromatophore matter/chromophore molecular complex with electroactive biopolymer similar to gum Arabica has been developed. In the work mentioned, variation bulk electrical conductivity with intensity and frequency of incident light on the developed complex was recorded. The electrical behaviour of this new complex is like that of an n-type semi-conductor. The chromaphoric light-harvesting (LH) molecules are capable of absorbing light, losing or gaining an electron even in the form of the mentioned complex. Light is used to induce charge separation [51]. The main function of the light-harvesting complexes is to gather light energy and to transfer this energy to the reaction centers for the photo-induced redox processes [52]. The solid specimens of the mentioned complex material were prepared and their electrical and photo-electrical characters were investigated experimentally. A brief description of the mentioned process is provided below.

Development of material: The chromophore molecules were extracted from flower petals by the following controlled process. The collected fresh flower petals were finely divided and heated in alcoholic double distilled water at 40°C to

bring chromatophores into the solution. The resulting solution was filtered and preserved. This solution was directly used to study the optical absorption. The filtrate was then allowed to form a complex with gum Arabica by the sol-gel process at 60°C at reduced pressure for six hours. The resulting sol was cast over an inactive substrate and allowed air-drying time to get it in the form of solid experimental specimens. In the study, chromophore molecules were used in the form of small clusters extracted from natural flowers of *China rose, Helianthus annuus* (Sunflower), and *Clitoria ternatea*. The molecules were employed to form complex systems with gum Arabica as a host EABP background matrix. The specimens developed were named (a), (b), and (c) respectively. The concentration of chromatophore matter in host gum Arabica was estimated at about 5% by weight. A more complex specimen (d) was developed using mixed (1:1:1) chromatophores from all three flowers to form a specimen that can absorb the entire region of visible spectrum. The role of gum Arabica was to provide a base matrix and preservatives. The properly dried specimens were employed for different electrical experimental analysis presented in the following section. The former guest molecules act as the light absorbing group and host material act as external donor or accepter. The developed photosensitive complex may be used in the development of solar cell.

Figure 12.37 shows optical absorbance of water suspension of extracted chromatophore from the above mentioned three flowers, in the UV-VIS region with water as the reference. All three chromatophores exhibit high absorbance in the UV region between wavelength 200–400 nm, a general feature of most natural biomolecules. The chromatophores from *Helianthus annuus* show a peak absorbance near 456 nm in the visible region and exhibit relatively lower absorbance in the luminous region of visible spectra, which is characteristic of the yellow colour of the natural flowers. *Chromatophores* from *Clitoria ternatea* show sharp absorption peak around 629 nm of visible region. The optical absorbance of chromatophores from the *China rose* shows a moderate absorption between 400–600 nm of visible spectra. The results of optical absorption for the last two natural chromatophores are also consistent with their natural deep blue and red coloured flowers respectively.

Figure 12.38 shows the optical absorbance of mixed chromatophores forming specimen (d) with water as reference. The result summarized in Figure 12.39 shows that the developed complex may be used to absorb light across most of the entire visible region. The said complex thus may be used for the development of a broadband photo-absorptive material.

Figure 12.39 shows the almost linear volt-ampere (I-V) characteristics of the illuminated solid Arabica complexes of the chromatophores specimens (a), (b), and (c) respectively, between applied p.d. 0–10 volt. Also shown is a comparison of the corresponding results obtained from measurement in near dark conditions. The nature of the graph shows that the current increases significantly in the presence of light, and calculated slopes of the respective curves show that in the presence of light the slope became almost half that of the dark measurement, i.e. electronic conductivities assumes about 100% enhancement due to photo induced charge separation in the specimens. In all cases the observed

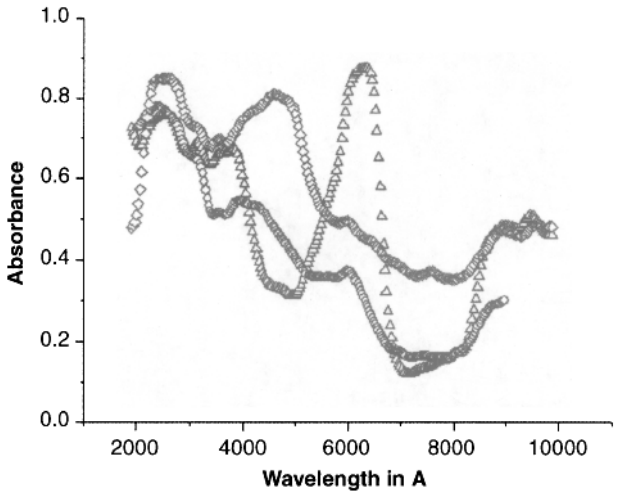

Figure 12.37 Optical absorbance of water suspension of extracted chromatophore from the mentioned three flowers, in the UV-VIS region with water as the reference [55].

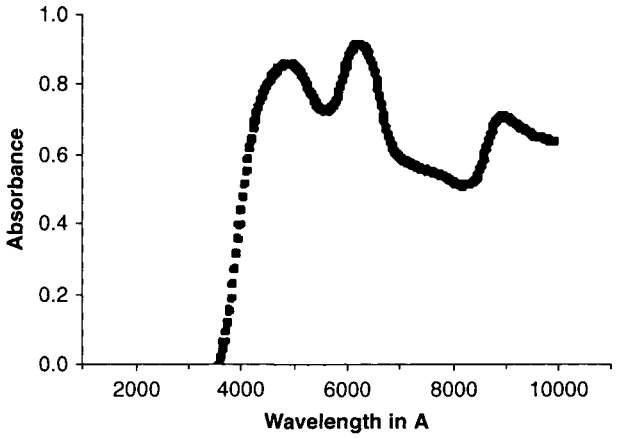

Figure 12.38 Optical absorbance of mixed chromatophores forming specimen (d) with water as reference [55].

photosensitivity is found to be very dominant. The developed material thus may be used for different types of photosensors.

Figure 12.40 shows the Cole-Cole plot of specimen (a), (b), and (c) respectively. In all three cases, the nature of the Cole-Cole plot is found to be a depressed semicircle over that of pure base matrix gum Arabica [17]. The Cole-Cole plot depicted indicates the nature of electroactivity in the complexes. The inclusion of micro/meso-sized chromatophore enhanced the electroactivity in the complexes to increase the electronic conductivity in them. Analysis of the Cole-Cole plot shows that it is a depressed semicircle showing overall EABP characteristics. The obtained semicircular impedance variation is more depressed compared to that of pure gum Arabica [17]. The depressed nature

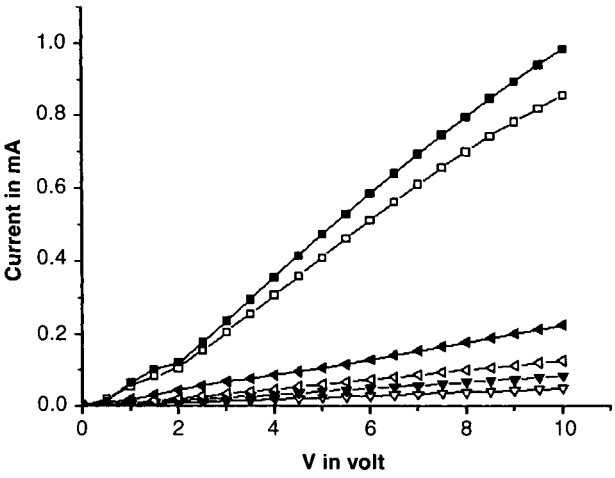

Figure 12.39 DC V-I characteristics of Chromophores and Gum Arabica complexes, (i) specimen (a) - ■ - with light and □- without light. (Sample thickness- 0.35 cm, Area- 2.25 sq cm). (ii) specimen (b) ▲- with light and Δ-.without light (Sample thickness- 0.25cm, Area- 2.25 sq cm) (iii) specimen (c) •- with light o- without light (Sample dimension same as (ii)) All records are at RT [55].

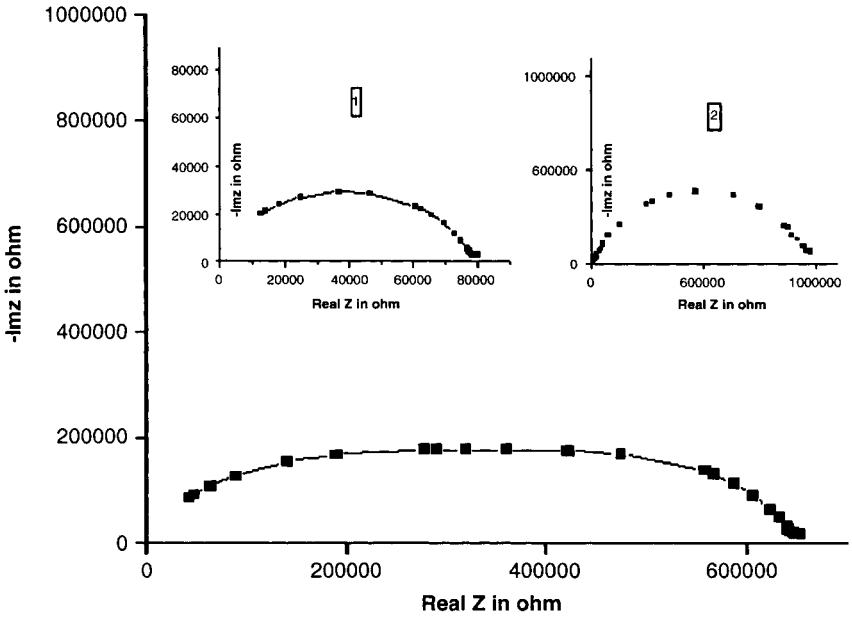

Figure 12.40 Cole-Cole plots – main, left inset 1 and right inset 2 curves are due to specimen (a), (b) and (c) respectively and dimensions Sample thickness- 0.35 cm, Area- 2.25 sq cm). (ii) specimen (b) ▲- with light and Δ-.without light (Sample thickness- 0.25 cm, Area- 2.25 sq cm [55].

is due to the inclusion of chromatophore molecule in the gum Arabica base matrix.

Table 12.4 compares the bulk total electrical conductivity of the developed specimens estimated from the measured complex impedance data. The table

also compares the respective electronic contribution from the results of d.c I-V characteristics of the respective specimens. It may be concluded that inclusion of chromatophores from natural flowers in the gum Arabica matrix enhances the electroactivity of the host matrix gum Arabica. The enhanced contribution of electronic conductivity is mostly due to inclusion of photosensitive chromatophore elements.

In another study [55], solid specimens of the LHC based gum Arabica complex were prepared, and the optical, electrical, and photoelectrical characters were examined experimentally. The chromophore/LH molecules used were in the form of small chlorophyll clusters from Sigma Aldrich (USA) and those extracted from the deep green leaf of the neem plant. The former was purified and expensive, whereas the latter was crude and cost effective. The optical, molecular, and photo-electric aspects of both developed specimens were compared, and extracted crude LHC was employed to form specimen (e), a complex system with gum Arabica as a host EABP background. The experimental results of the above mentioned study are summarized below.

The optical absorption of pure chlorophyll and the extracted crude variety are compared and shown in Figure 12.41. Graph A and B represent the

Table 12.4 Comparison of ionic and electrical conductivity of chromophore complexes of Gum Arabica.

Different Chromophore with Gum Arabica complex	Total Conductivity (Ionic + Electronic) [S/cm]	D.C Conductivity (Electronic) [S/cm]
Specimen (a)	$7.85*10^{-5}$	$1.63*10^{-5}$
Specimen (b)	$7.5*10^{-6}$	$9.5*10^{-6}$
Specimen (c)	$5*10^{-7}$	$4.5*10^{-7}$

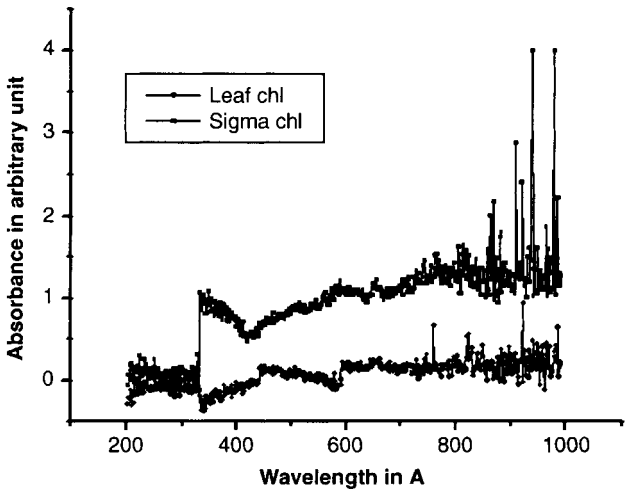

Figure 12.41 Comparison of optical absorbance between natural chlorophil collected from leaf (open circle) and chlorophil (dots) collected from Sigma Aldric [55].

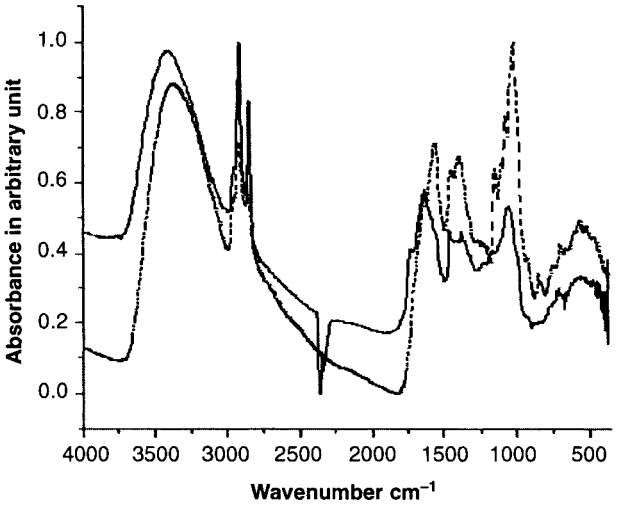

Figure 12.42 FTIR absorbance of pure chlorophyll _____ full line and of crude chlorophyll ------- lines [55].

absorbance of pure chlorophyll (from Sigma Aldrich) and that of extracted crude specimen respectively. Their overall nature, including the characteristics absorbance, peaks around 440 nm and 660 nm and are similar except for the difference in optical densities. The results obtained are comparable to that reported by Lichtenthaler and Buschmann [55] except for the difference in background due to the use of different reference.

The results of FTIR analysis on chlorophyll LHC complex from Sigma Aldrich (USA) is shown in Figure 12.42. The figure also compares IR absorption from crude chlorophyll complex extracted from neem leaves. The comparison between their nature of IR absorption indicates that the molecular composition of the specimens are mostly the same, and that crude extracted chlorophyll can be used in cost effective ways for nonbiological applications such as light-harvesting or development of solar cells.

12.8 Development of Biopolymeric Solar Cells

Conventional solar cells are built from inorganic silicon-like materials. Efficiency of such solar cells is high, but they originate from expensive materials and special techniques are required for their processing. Recently hybrid and photoelectrochemical solar cells [54] have been cost effective alternatives for conventional silicon solar cells. The correspondence between the photon absorption and charge separation events is the point of differentiation between the photovoltaic effect in a semiconductor junction, and that in a photon-induced generation of a chemical potential in natural systems, i.e. photosynthesis. In the latter, and this is very simple but highly relevant in the context of artificial photosynthetic systems, the point in space at which the

primary light absorption event occurs can be very different from the point at which the effect is ultimately felt through the generation of spatially separated charge carriers. The photon absorption leads to the production of electricity depending upon device design, but that field need not exist at the absorption site. The key advantage of this mechanism is that photon absorption and charge separation can be decoupled and therefore different molecules.

LHC-based, nano-implanted complex specimens were also developed using ZnO and TiO_2 nanocomplex of gum Arabica and chlorophyll LHC molecules by the chemical sol-gel route. The ZnO and TiO_2 nanocomplex of gum Arabica were prepared following natural self-assembly of ZnO and TiO_2 nanoclusters in gum Arabica complex of LHC background. The later was found [42] to be a good surfactant or capping medium for the nanoclusters. The specimens thus developed were specimen (f) ZnO implanted and specimen (g) TiO_2 implanted complexes respectively. All three complex specimens, (e), (f) and (g), were found to exhibit properties like that of n-type semiconductors, while the complex specimen (h), analogous to the p-type semiconductor, was developed by iodine (I2) doping in the developed complex specimen (e). The solid specimens (f), (g) and (h) of the above mentioned nanocomplex were employed to develop non-silicon based hetero-junction type solar cells.

Figures 12.43 and 12.44 shows the TFM picture of the developed ZnO and TiO_2 nanoclusters in gum Arabica background. The same nanocomposites

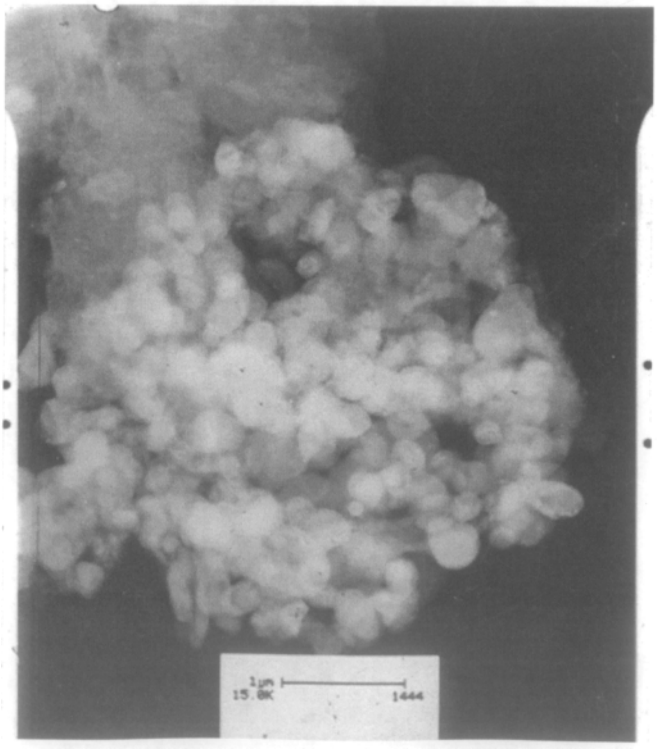

Figure 12.43 TEM picture of the developed ZnO in Gum Arabica background [55].

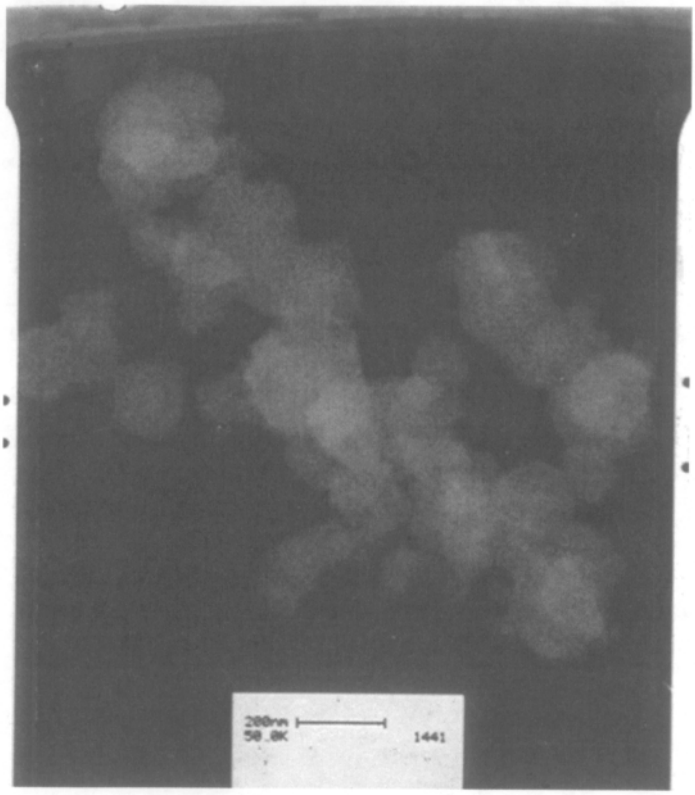

Figure 12.44 TEM picture of the developed TiO_2 nano-clusters in Gum Arabica background [55].

were used to develop the nano-implanted LHC complex for the development of the solar cell. The average cluster sizes grown therein were about 40nm and 80 nm respectively. The nanoclusters of ZnO and TiO_2 were implanted in the specimen (f) and (g).

The I-V characteristics of the developed non-silicon based biopolymeric solar cell [55] are shown in Figure 12.45. In the developed chlorophyll, a complex of gum Arabica with a small proportion of gum mangosteen provides the n-type layer, and that with iodine composite constitutes the p-type layer of the heterojunction type solar cell. The developed cell used the prolong function of LH molecules in EABP background. Another type of solar cell [55] is developed following that of a hybrid type cell. A hybrid solar cell consists of both organic and inorganic materials and therefore combines the properties of inorganic semiconductors with the film forming properties of the conjugated polymers. Organic/biomaterials are low cost, easily processed, and their functionality can be tailored by molecular design and chemical synthesis. Nanoparticles offer the advantage of having high photo absorption coefficients and size tunability. By varying the size of the nanoparticles, the cell function can be tuned and therefore the absorption range can be tailored. The solar cell mentioned was fabricated using the developed material which is a ZnO nanocomplex

of chlorophyll complex of gum Arabica with gum mangosteen sandwiched between a redox-pair (Cu-Zn as the simplest choice) o f electrodes without any heterojunction. The ZnO nanocluster in the composite enhances the photo absorption, and the energy transfer process in the complex leads to high performance of the developed solar cell. Figure 12.46 shows the action of such a solar cell [55].

The supramolecular complexes developed in this work consist of a light absorbing component, chromophores, and the background host is a large molecular natural biopolymer gum Arabica [17]. The developed solid specimens are

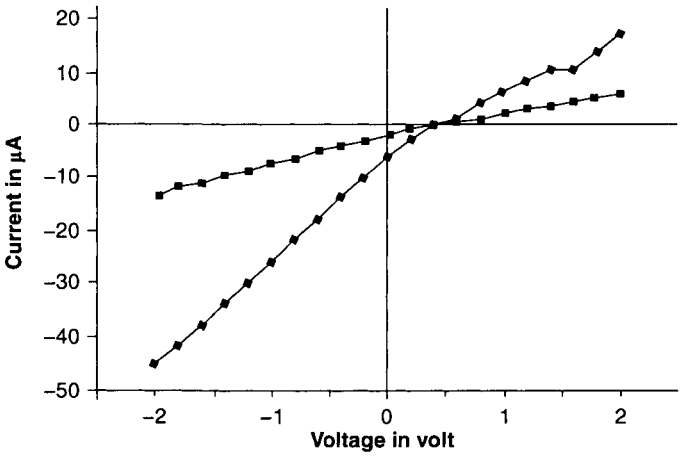

Figure 12.45 I-V characteristics of the developed photo-sensitive complex of Gum Arabica in light (♦) and in low light (■) [55].

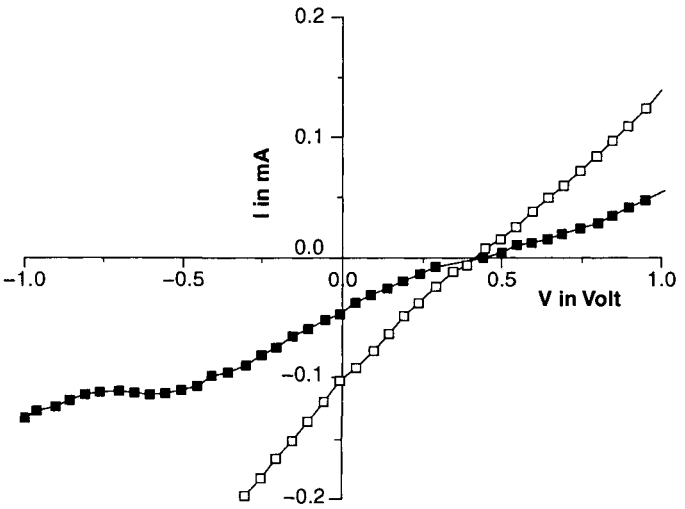

Figure 12.46 Characteristics of junction made with ZnO nano complex of Gum Arabica and iodine complex of the Gum sample, showing low light (■) and with light (•) [55].

capable of absorbing light to induce events such as charge separation, initiate catalysis, and interrogate the system in sensing applications. The inclusion of photochemically active component within the supramolecular complex may be expected to perturb or modulate the photochemical behaviour of the chromophores, producing effects such as photo induced charge separation, energy transportation, charge in optical transitions, and polarisabilites. The photoabsorption process also introduces modification in ground or excited state redox potential and selective photochemical reactivity in the specimen. The overall observation implies that the photosensitivity in the developed complexes is originating from chromatophore molecules of natural flower/leaves. Recently, the role of light-harvesting molecules in explaining the photosynthesis process in plant leaves has been investigated [56, 57]. However, the role of LH molecules in the other plant parts, namely flowers and roots, have been paid little attention. It is apparent that the light-harvesting complex (LHC) occurs mainly in the thylakoid stack of choloroplast grana where it appears to be associated with the chlorophyll-protein complexes of photo-system. This study leaves the strong impression that LHC molecules in chromoplast of plants perform LH action in other parts and organs of the body. The potential of LHC molecules can be exploited in developing technological applications [58].

The developed specimens (f) and (g) are n-type in nature, whereas specimen (h) is p-type. The prototype solar cells developed and discussed here are (i) p-n type heterojunction; (ii) unipolar with redox pair of electrodes. I-V characteristics of the developed biopolymeric solar cell are shown in Figure 12.47. Figures 12.47a, 12.47b, and 12.47c show the I-V characteristics of the developed solar cells, namely HJ1, HJ2, and HJ3 respectively. In all cases, the recorded current density was found to be high along with the scope for further modification. The short circuited current and open circuit voltages of all the cells are given in their respective figure captions. It appears that all the developed cells are competitive.

The developed chlorophyll complex of gum Arabica with small proportion gum mangosteen [56a] provides the n-type layer, and that with iodine composite constitutes the p-type layer of the planer heterojunction type solar cell. The optical absorption of pure mangosteen gum [59] over the wavelength region 190–900 nm is fascinating to see. The nature shows a very high absorbance between 350–900 nm, i.e. over the entire visible region along with a small region on either side of the VIS region. The material may be used as a light absorbing layer over the planer solar cell. I-V characteristics of the developed proto-photocells were investigated under dark and illuminated conditions. It showed a drastic change in the cell current along with a relatively small change in the cell emf due to irradiation of the specimen. This simple photocell performance is an indicator of the potential use of the developed chromatophore complex toward the development of a non-silicon-based solar cell and light-harvesting process. Recently [60–62], non-silicon type solar cells appeared to be very important due to their inherent characteristics. Dye-sensitized solar cells (DSSC) [60–62] have been recently reported, however, they are not cost effective compared with silicon cells. In this regard the developed LHC-based solar cells are a non-silicon type. Their novelty lies with their cost effectiveness

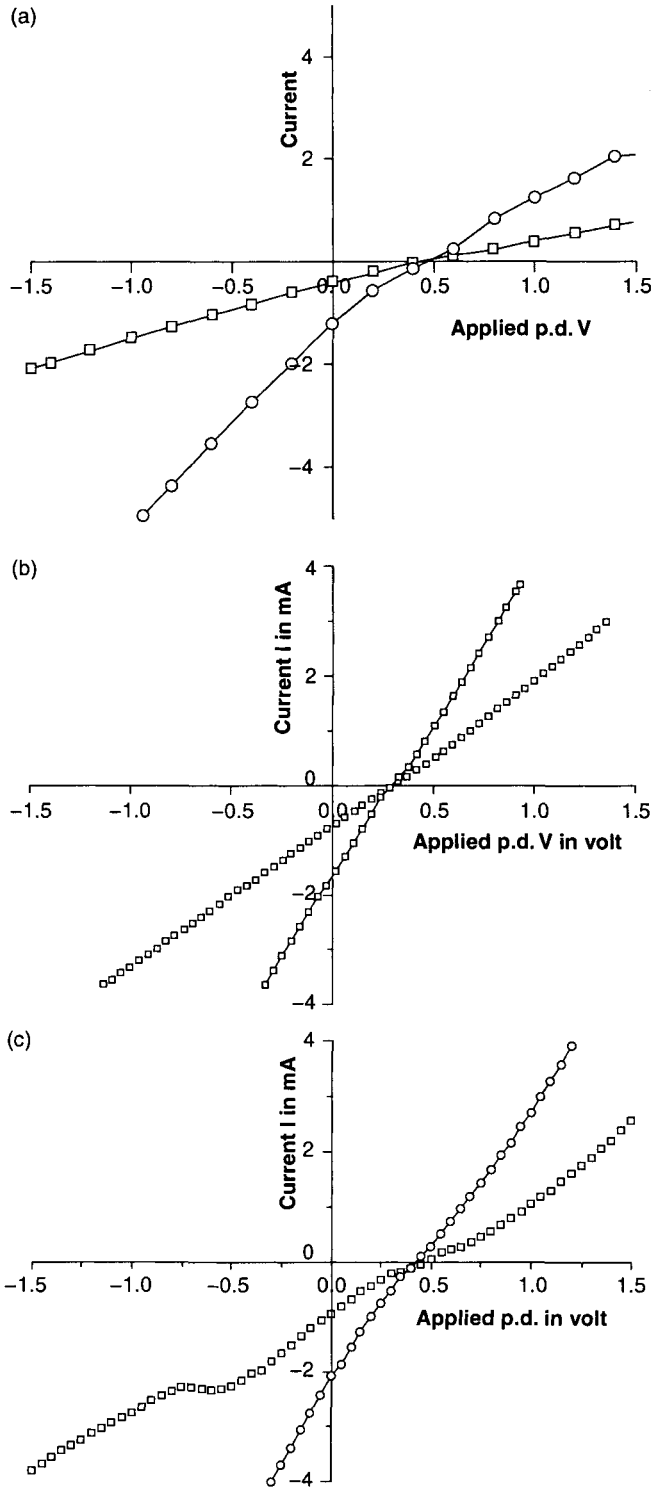

Figure 12.47 a-c V-I characteristics of prototype solar cells HJ1, HJ2 and HJ3 respectively with Cu-Cu electrode, under very low light (□) and with sunlight(○). (All are with Sample thickness- 0.08cm, Area- 1.1 sq cm at RT) [55].

and all green nature. It is apparent that current density of the developed cells may be improved further by lowering the junction thickness and reducing the size of the doped nanoclusters.

A unipolar type of solar cell is developed following that of hybrid type cells. A hybrid solar cell consists of both organic and inorganic materials, and therefore combines the properties of inorganic semiconductors with the film forming properties of the conjugated polymers. Organic biomaterials are low cost, easy to process, and their functionality can be tailored by molecular design and chemical synthesis. Nanoparticles offer the advantage of having high photoabsorption coefficients and size tunability. By varying the size of the nanoparticles, the cell function can be tuned, and therefore the absorption range can be tailored. The mentioned solar cell has been fabricated using the developed specimen (e), (f) and (g), of which the latter two are a ZnO and TiO_2 nanocomplex of chlorophyll complex of gum Arabica with gum mangosteen. The specimens were sandwiched between a redox pair (Cu-Zn as the simplest choice) of electrodes without any heterojunction. ZnO and TiO_2 nanoclusters in the composite enhance the photo absorption and energy transfer process of the developed complex. In fact, it leads to the development of high performance non-silicon type solar cells. It has been observed that the addition of a small amount of polyvinyl alcohol (PVA) in all the developed complexes provides better mechanical strength in the form of a thin film without affecting the photoelectric properties. Figures 12.48a, 12.48b and 12.48c show the I-V characteristics of unipolar type solar cells UJ1, UJ2, and UJ3 respectively. All the results show an efficient performance of the cell with promising efficiency and high current density. In fact, the overall efficiency thus obtained is better compared to that of DSSC-type solar cells. Figure 12.48a shows I-V characteristics of a unipolar solar cell using specimen (e). This is again a good result for an all green type of approach; however, its overall performance is slightly subdued compared to those obtained for UJ2 and UJ3.

12.9 Biomedical-like Application

Gum Arabica is an eco-friendly, nontoxic plant gum with high material potential as discussed in the previous sections. The gum has medicinal potential which has been established from results of earlier workers in the field. In this section an application of gum Arabica aiming at drug delivery will be highlighted. In a recent development [63], nanosized ZnO clusters were developed in the gum Arabica background. The latter is a good surfactant agent that provides good capping to ZnO nanoclusters. In bacterial growth for example, yeast gum Arabica can be used merely as a carbon source, i.e. it neither helps nor resists the growth of yeast. In a very recent analysis [64] it has been observed that when the ZnO nanocomplex of gum Arabica was delivered on grown yeast, the ZnO nanoclusters embedded on the bacteria surface. Yeasts are of much interest in research, both in their multi-variant role as pathogens and as biotechnical important organisms, and as model systems for eukaryotic

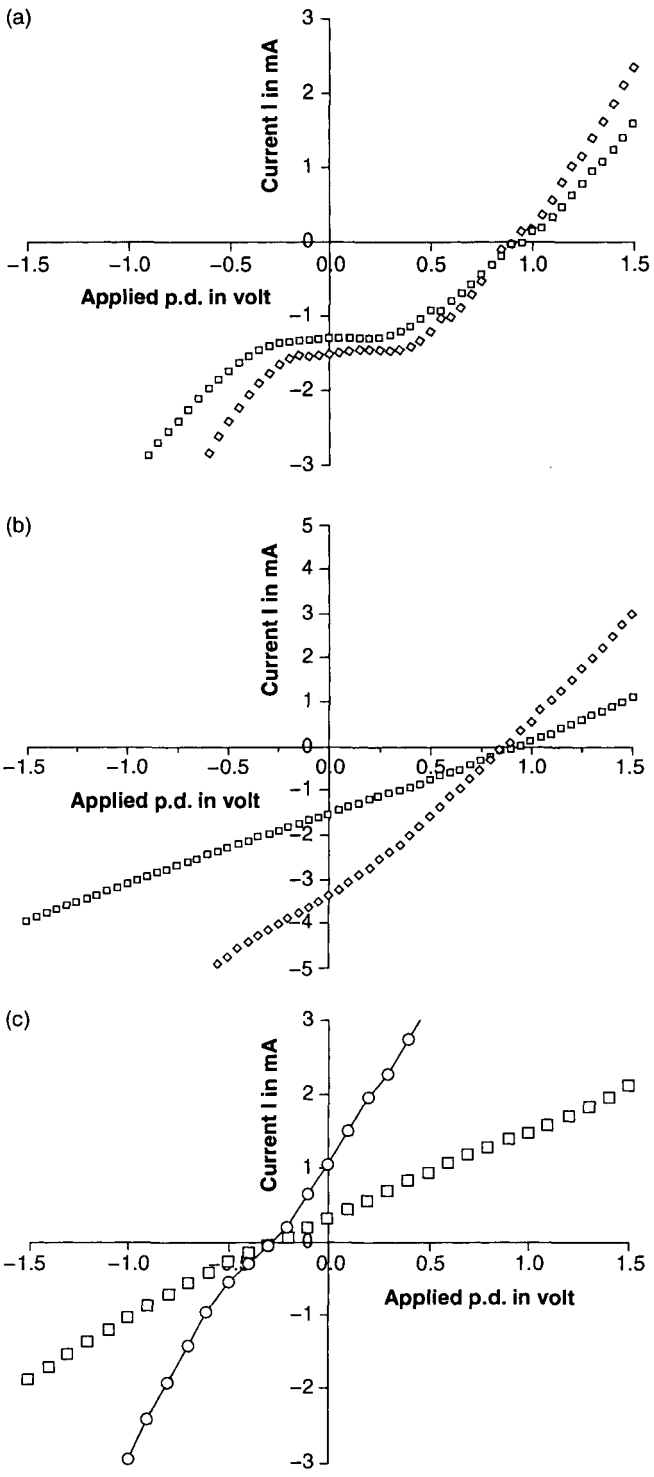

Figure 12.48 a-c V-I characteristics of proto solar cells UJ1, UJ2 and UJ3 respectively with Cu-Cu electrode, under very low light (□) and with sunlight (○). (All are with Sample thickness- 0.08 cm, Area- 1.1 sq cm at RT) [55].

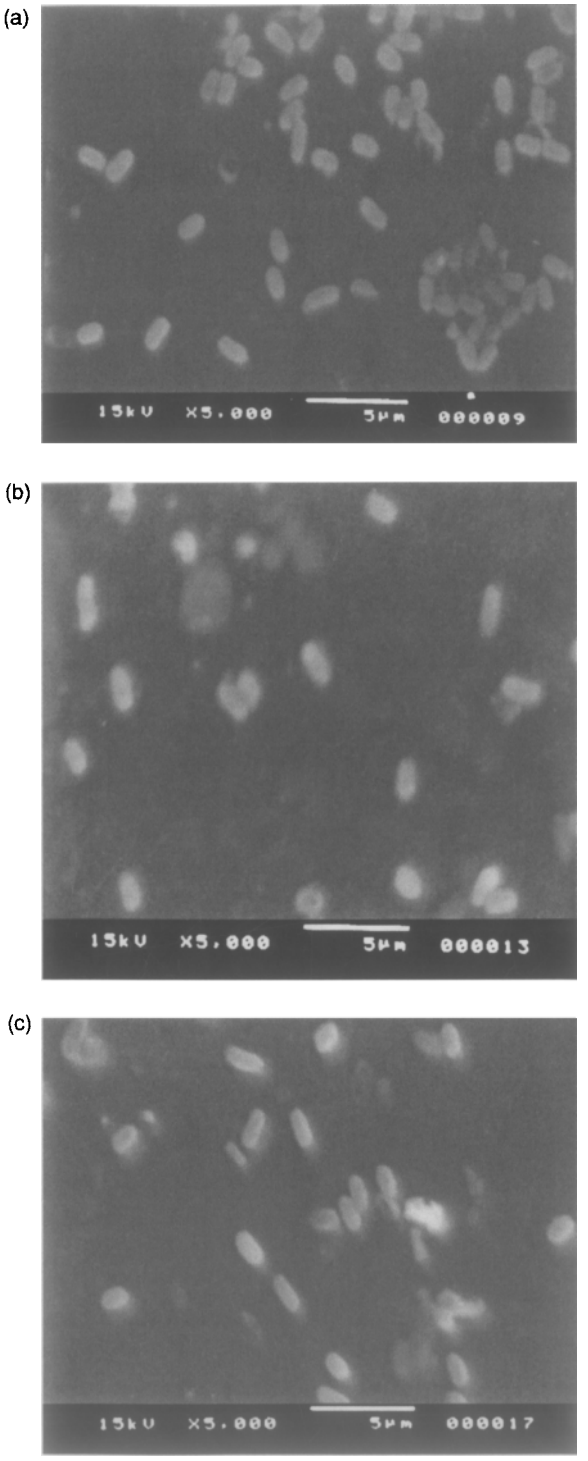

Figure 12.49 a-c SEM picture yeast cells grown in Gum Arabica background (a) pure yeast (b) yeast + ZnO (c) yeast + ZnO after LASER irradiation [64].

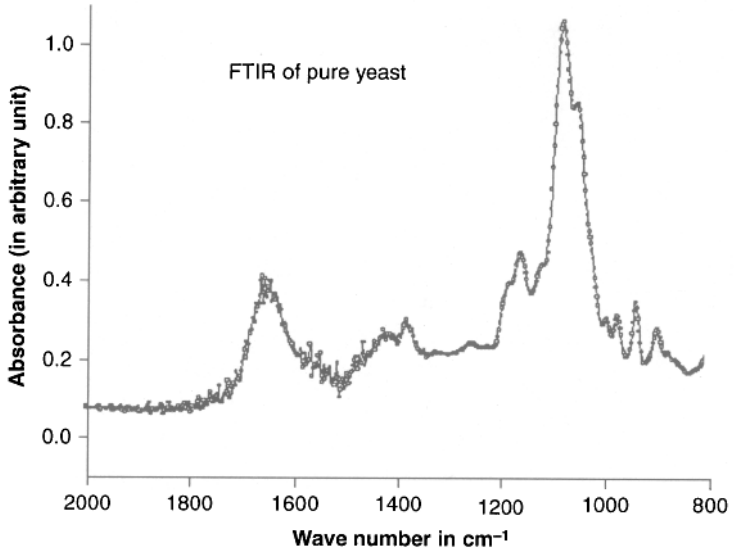

Figure 12.50 FTIR absorption spectrum of pure yeast cells [64].

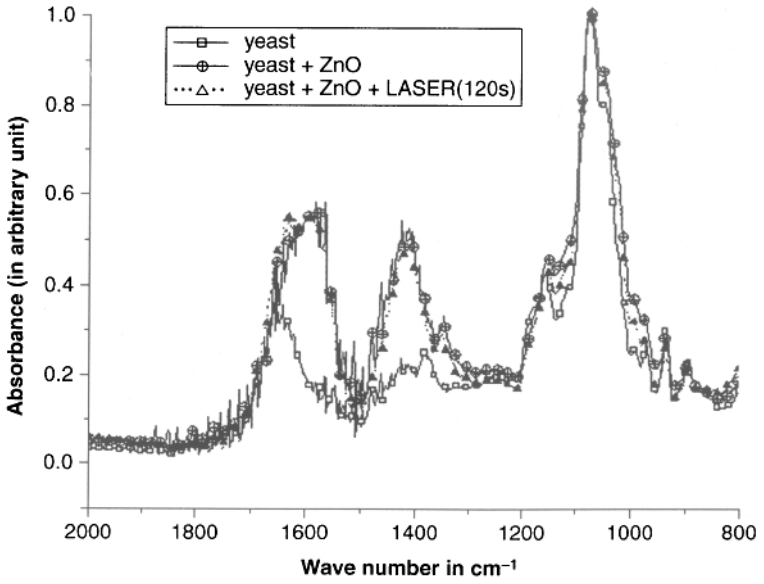

Figure 12.51 Comparative FTIR absorption spectrum of yeast cells in Gum Arabica based ZnO nana – complex medium and LASER irradiated specimen of the same [64].

cells. In particular, much attention has been paid to *Saccharomyces cerevisiae* for use in several studies [65]. In the mentioned work [64] a sample was prepared using YPDA medium with ZnO particles as a nutrient medium for the yeast (*S. cerevisiae*) strain to grow. Another sample, sample-III was prepared allowing yeast to grow simply on YPDA medium at a particular incubation temperature using the streaking procedure. YPDA medium was autoclaved to

remove any contamination. For yeast growth, 24–25°C incubation temperature and 3–4 days' time was allowed. ZnO nanoparticles were prepared capping with Acacia gum and ethanol (CH_3CH_2OH) respectively.

The SEM micrograms, shown in Figure 12.49a, 12.49b and 12.49c respectively, show the effect of the addition of ZnO nanoclusters on the yeast bacteria via gum Arabica. The inclusion of ZnO nanoclusters over yeast bacteria was also verified from FTIR analysis. Figure 12.50 shows the comparative FTIR absorption of pure yeast with some that have ZnO nanoclusters inflected bacteria. A laser irradiation on the ZnO nanoclusters inflected yeast bacteria showed a possible destruction of the bacteria. The result of laser irradiation is summarized by the FTIR absorption spectra shown in Figure 12.51. This preliminary work is an *in vivo* application of drug delivery principle using gum Arabica. Even more uses exist for the biomedical application of gum Arabica.

12.10 Conclusion

Gum Arabica is an electroactive biopolymer, and this conducting biomaterial has a substantial ionic contribution like other solid protonic conductors. Its electroactivity can be tailored by a great extent through formation of their complexes with appropriate organic and inorganic dopant. The overall material characteristics of those investigated so far are very interesting. The presence of an organic acid unit in the molecular structure provides high electrical conduction. The inclusion of a photo-absorbing group induced charge separation provides high carrier concentration. The photo induced charge separation is seen in many biomaterials, and the property has also been actualized in the development of photosensitive biomaterial complex. The inclusion of nanocluster is found to be more effective than that of organic natural acid. The former enhance photo-absorption, and the latter require adequate activation to generate carrier. The surfactant property of gum Arabica is very good for developing and analyzing nanoclusters. The electrochemical property of gum hydrogel is also very important.

Acknowledgements

The author acknowledges U. G. C., New Delhi, India for the support of this research M.R.P. NO. F-10-5/98(SR-I) &. (No. F30-2/2004 SR). The author is also thankful to senior students, Dr. H. Mallick, Dr. S.S Pradhan, and Miss. Prapti Mukhopadhyay, and the students. The author would also like to acknowledge SAIF, IIT Bombay, and UGC-DAE-CER, Indore, India.

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13

Gluten: A Natural Biopolymer

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Abstract

Biopolymers are a diverse and remarkably versatile class of materials that have potential applications in virtually all sectors of modern industrial economics. Cereal grains can be considered as an agricultural raw material rich in several natural polymers (biopolymers). Cereals are the most important crops in the world, with total annual grain yields exceeding 2000 million tons (mt). Although a number of cereal species are grown, the three that together account for over 70% of the total production are maize (604 mt in 1998), wheat (589 mt in 1998) and rice (563 mt in 1998). Other cereals including barley, sorghum, millets, oats and rye, also have an important impact on humans and livestock. The total protein contents of cereal seeds vary from about 10–15% of the grain dry weight, with about half of the total being storage proteins. Nevertheless, proteins have a major impact on the end-use properties of the grain.

Osborne classifies seed proteins into three groups: storage proteins, structural and metabolic proteins, and protective proteins. Triticeae seed storage proteins fall into four groups:

- Albumins – soluble in hypotonic solutions and are coagulated by heat
- Globulins – soluble on isotonic solutions
- Prolamins – soluble in aqueous alcohol
- Glutenins – soluble in dilute acids or bases, detergents, and chaotropic or reducing agents.

Of particular importance are the glutenin polymers, and it is well established that strong doughs contain high proportions of high molecular mass glutenin polymers. HMW subunits of glutenin strongly correlated with differences in the breadmaking quality of bread wheats. Bread wheat has six HMW subunit genes on the long arms of the group 1 chromosomes (1A, 1B, 1D), each locus comprising two genes encoding subunits that differ in their properties and are called x-type and y-type subunits. Good breadmaking quality is particularly associated with the presence of a 1Ax subunit and the chromosome 1D-encoded subunit pair 1Dx5 + 1Dy10.

The storage proteins of cereals are of immense importance in determining the quality and end use properties of the grain. Understanding the structure of these proteins,

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (377–402)

their genetical, biophysical and functional properties, are important to underpin future attempts to improve the end-use quality of grain by genetic engineering.

Keywords: Wheat, maize, barley, rye, gluten, gliadin, glutelin, HMW, LMW

13.1 Introduction

Most plants synthesize a type of protein whose main function is to provide a store of nitrogen, sulphur and carbon. The majority of these storage proteins are synthesized in organs of reproduction, propagation and dispersal, such as seeds, pollen, spores and tubers. Seeds of cereal plants contain large amounts of storage proteins, most of which fall into the prolamine and globulin class. Prolamines are the major seed storage proteins in all cereals except oats and rice. Wheat is the most widely grown cereal in the world. The production is around 580 million per ton (2001) (Figure 13.1).

Gluten (from the Latin *gluten* "glue") is the composite of a prolamin and a glutelin. These exist, conjoined with starch, in the endosperm of some grass-related grains, notably wheat, rye, and barley. Gliadin and glutenin (the prolamin and glutelin from wheat) comprise about 80% of the protein contained in wheat seed. Worldwide, gluten is a source of protein, both in foods prepared directly from sources containing it, and as an additive to foods otherwise low in protein. The seeds of most flowering plants have endosperms with stored protein to nourish embryonic plants during germination (Figure 13.2).

The term gluten is also used in commerce (erroneously) to indicate the protein residue after isolating starch from corn (maize) [1]. However, this 'corn gluten' is functionally very different from wheat gluten. Another connotation of the term 'gluten' relates to the family of proteins that cause dietary problems for people with celiac disease [2]. In this case, the term 'gluten' includes the storage proteins from the grains of rye, triticale, barley and possibly oats [3].

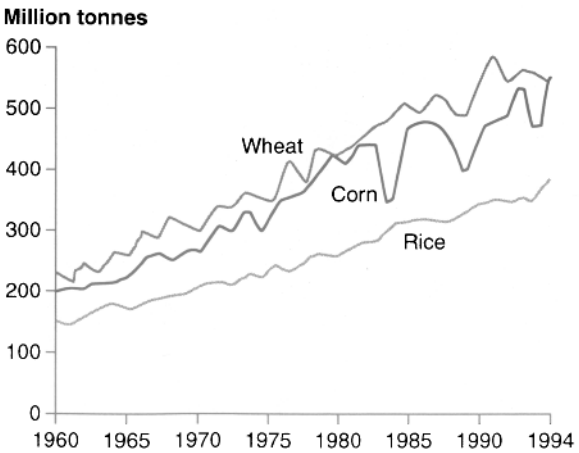

Figure 13.1 World Production of Wheat, Corn and Rice.

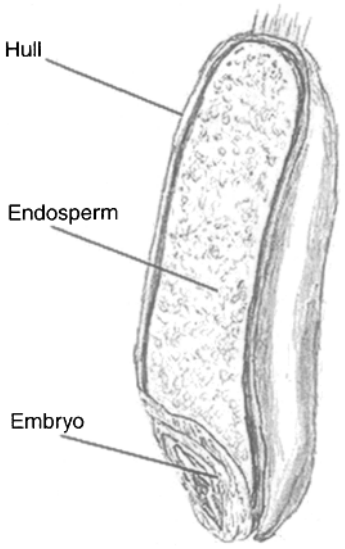

Figure 13.2 Wheat seed, endosperm and embrio.

Thus, the term 'gluten-free foods' refers to food products free from these cereal proteins, or those in which cereal protein content is less than a defined amount (usually 200ppm).

Wheat endosperm proteins were among the first proteins to be studied when Baccari in 1745 reported the isolation of gluten. Later, storage proteins from rye and barley were isolated. At the beginning of the 20th Century, a systematic study was conducted by Osborne (1907) to develop a classification for cereal seed proteins based on their sequential and differential solubility. He classified wheat proteins into four different groups, albumins (soluble in water and delute buffers), globulins (not soluble in water but soluble in saline solutions), prolamins (which are soluble in 70–90% ethanol), and glutelins which are soluble in dilute acid or alkali). Chen and Bushuk (1970) [4] added a fifth fraction to the original four from Osborne's procedure. It came from dividing glutenin into two fractions: one soluble in dilute acid (0.05 M) and the other insoluble in this solvent. The majority of the seed proteins are stored in the starchy endosperm in the form of prolamins, which are unique to cereal grains, and account for over half of the total seed nitrogen. Prolamins in general are known for their nutritional qualities, but they are not only associated with functional quality in wheat.

Although Osborne's fractionation was a major milestone in the development of cereal chemistry, it must be understood that each of these fractions is a complex mixture of different polypeptides, and also that these polypeptides overlap in their solubilities. This is particularly true for the gliadins and glutenin proteins. Beckwith *et al.* (1966) detected by gel filtration studies a small fraction of the ethanol-soluble proteins as an excluded peak, which he called gliadin of high molecular weight (HMW) [5]. Nielsen *et al.* (1968) found that

these proteins behaved like glutenins in showing a drastic decrease in viscosity after reduction of disulfide bonds, and therefore called them glutenins of low molecular weight [6]. Later, Jackson *et al.* (1983) using 2D electrophoresis showed that the constituent subunits of this fraction were identical to LMW-GS and different from the gliadin polypeptides [7]. Field *et al.* (1983), using 50% 1-propanol instead of 70% ethanol, also found some HMW-GS along with the LMW-GS (GS glutenin subunits) within the alcohol-soluble fraction. In any case, after the reduction of disulfide bonds, all gluten proteins are solube in 70% ethanol or other alcohol such as *n*-propanol [8]. Shewry *et al.* (1986) classified them as prolamins [9]. In addition, the existence of close similarity in structure between LMW-GS and gliadins is another reason to consider them within the same group (prolamins). Within this group, further differences between them are based on biochemical characteristics (sulfur-rich prolamins including α-, β-, and γ-gliadin and LMW subunit of glutenin, sulfur-poor prolamin including ω-gliadins, and HMW prolamins (Figure 13.3).

The sulfur-rich prolamins are quantitatively the most abundant group in wheat, barley and rye, accounting for approximately 80–90% of total prolamin fractions. They include polymeric and monomeric components and consist of at two families in each species, the B- and γ-hordeins of barley; two types of γ-secalin of rye; and α, β and γ-gliadins and LMW glutenin subunits of wheat. The sulfur-poor prolamins include C-hordeins of barley, ω-secalin of rye and ω-gliadin of wheat.

Ewart (1990) [10] criticized Shewry's classification, arguing that the polymeric glutenins are fundamentally different from the monomeric gliadins because of their intermolecular disulfide bonding capacity [11].

Wheat gluten consists of a complex mixture of proteins, which are classically divided into two groups [12] (Figure 13.4). A major difference between these two groups of storage proteins is found when analyzing their functionality. While gliadins are single polypeptide chains (monomeric proteins), the

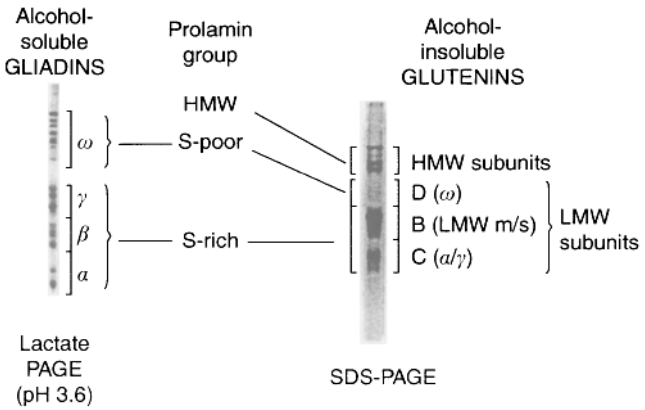

Figure 13.3 The classification and nomenclature of wheat gluten proteins separated by SDS-PAGE.

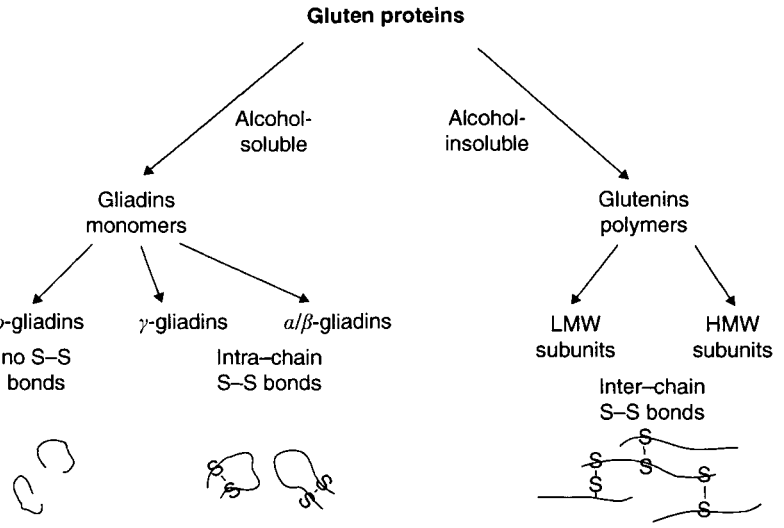

Figure 13.4 The major groups of wheat gluten proteins. The ω-gliadins and HMW subunits are defined as S-poor prolamins and HMW prolamins respectively. The α/β-gliadins, γ-gliadins and LMW subunits are S-rich prolamins [12].

glutenins are multichained structures of polypeptides that are held together by disulfide bonds. The very high molecular weight of these polymeric structures is responsible for their partial insolubility and for their distinct contribution to functionality compared with that of the gliadins. The HMW-GS, which represent approximately 0.5% of the total seed dry weight, have been studied extensively because of their effect on elasticity, and hence the breadmaking quality of wheat dough.

During the mixing of wheat flour with water to make dough, the prolamins form the gluten, a continuous proteinaceous network that is the basis of dough functionality. The wheat prolamins are divided on the basis of function into two groups, the glutenins and gliadins, which together confer the properties of elasticity (strength) and extensibility (viscosity). Gliadins and glutenins are the seed endosperm prolamin proteins. These unique properties of wheat gluten are not found in the storage proteins of other cereals and are the basis of the wide range of food products derived from wheat.

Seed storage proteins of grain crops meet the major dietary protein requirement of over half of the world population. However, seed proteins in general are deficient in some essential amino acids and are of poor nutritional quality (Table 13.1).

Lysine is the first nutritionally limiting essential amino acid in most cereals (1.5–4%); tryptophan (0.8–2%) is the second limiting amino acid in maize, and threonine (2.7–3.9%) in other cereals. The high content of prolamins in cereals is responsible for the low content of essential amino acids like lysine, threonine, valine and isoleucine. Rice and oats have a better balance of essential amino acids than other cereals due to a lower content of prolamins.

Table 13.1 Contents of essential amino acid in grain of wheat, rye and barley compared with the WHO recommended levels (FAO, 1973).

Amino acids	Wheat	Rye	Barley	WHO Recommended Values
Lysine	2.0	3.3	3.1	5.5
Methionine	1.3	1.7	1.7	3.5
Tryptophan	1.1	1.8	2.0	1.0
Valin	3.7	4.4	4.6	5.0
Phenylalanine	5.1	4.9	5.5	6.0
Histidine	2.2	2.1	1.9	-
Cysteine	2.6	2.9	2.9	3.5
Leucine	6.7	6.7	7.2	6.0
Threonine	2.7	3.4	3.3	4.0

Plant geneticists and breeders have made significant effort in the past to improve the quality of seed proteins. High lysine content (up to 4.0% average per 5 years) and tryptopnan (0.9%) mutants have been obtained in T. durum. A new allele b/band 2*1* of Glu-A1 locus was described [13] (Figure 13.5).

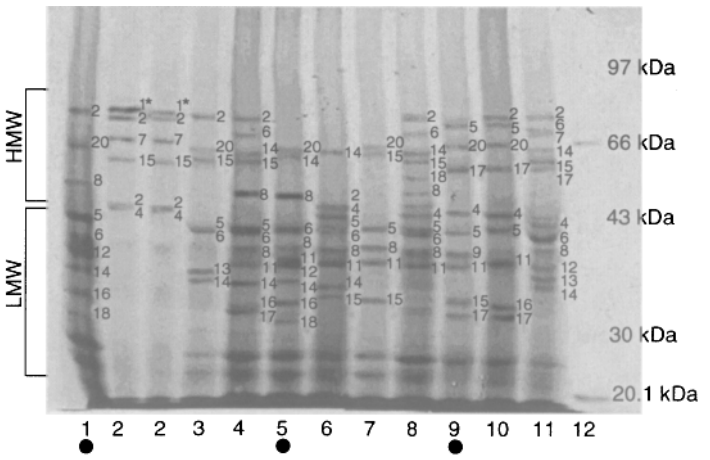

Figure 13.5 Allelic variation in 4x wheats and their mutant forms. SDS-PAGE patterns of total seed proteins from: (1 and 5) T. durum Des. And T. durum 788-control forms, (2, 3, 4, 6, 7, 8, and 9 mutant forms type sphaerococcum). Lane 10 and 11 – T. aestivum. 12-LMW standart [13].

13.2 Gliadins

Gliadins are a heterogeneous mixture of single-chained polypeptides which are, in their native state, soluble in 70% aqueous alcohol. In accordance with their mobility in acid-PAGE, as a monomeric protein gliadins are divided into four groups: α- (fastest mobility), β-, γ-, and ω- (slowest mobility) gliadins. Molecular weight range is ≈30 000 to 80 000 Da. After one-dimensional electrophoresis, gliadins from single wheat grain can be separated into 20–25 components [14]. Two-dimensional electrophoresis allows better separation with a resolution of up to 50 components [15].

The γ-gliadins differ from α- and β-gliadins in the amount of aspartic acid, methionine, tyrosine, phenylalanine, and tryptophan [16]. The ω-gliadins differ in amino acid composition from other gliadins and do not have cysteine. The ω-gliadins are characterized by high levels of glutamine (40–50 mol %), proline (20-30 mol %), and phenylalanine (7-9 mol %), which represent >80% of the total amino acid residues. Also, gliadins can be classified according to their N-terminal amino acid sequence.

The prolamins of wheat are highly polymorphic polypeptide mixtures of components with M, values ranging from 30,000 to 90,000 [17]. Wheat endosperm proteins are heterogeneous. For example, at least 46 gliadins exist [18], most of them are monomers of 30,000-40,000 mol wt (α-, β-, and γ-gliadins) or 60,000-80,000 mol wt (ω-gliadins) [19] (Figure 13.6) [20], but Sephadex G-100 chromatography also reveals 5–10% of a 100,000-125,000 mol wt fraction.

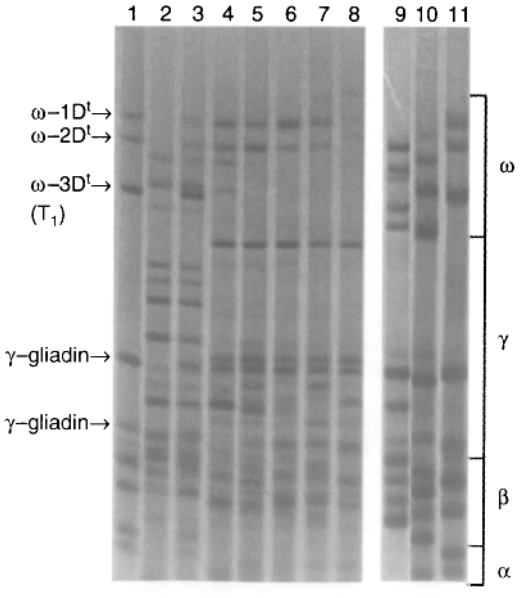

Figure 13.6 A-PAGE analysis of gliadins. Lanes: 1, T. tauschii accessi 4-8, T. aestivum cvs Kite, RAC704, RAC746, Meering, Cheyenne; 9, T. tauschii accession CPI 110750; 10, T. tauschii accession CPI 110856; and 11, T. tauschii accession AUS 18913 [20].

In contrast, the glutenins form large polymeric structures as a result of intermolecular disulfide bonds.

True gluten, with gliadin and glutenin, is limited to certain members of the grass family. The stored proteins of maize and rice are sometimes called glutens, but their proteins differ from wheat gluten by lacking gliadin. The glutenin in wheat flour gives kneaded dough its elasticity, allows leavening and contributes chewiness to baked products like bagels and flour tortillas. Wheat is the number one food crop in the world based on area under cultivation and total production. Wheat is considered a staple crop because of its unique superiority for making leavened bread. This is due to the viscoelastic properties of its storage protein-rich gluten polymer, which is obtained following hydration of the n wheat flour. Although all wheat seed storage proteins are part of the gluten, the glutenin polymers are considered the most important determinants of its viscoelastic property.

It should be noted that prolamins are also classified into three groups, which do not correspond to the more validly used gliadin/glutenin classification [9]. Two of these groups correspond to the ω-gliadins (The S-poor prolamins) and the HMW subunits of glutenin (the HMW prolamins) respectively, while the third group (the S-rich prolamins) comprises gliadin (γ- and α/β-) and glutenin (LMW subunit) components. The amino acid sequences of typical members of these groups are summarized in Figure 13.7 [12, 21].

All wheat prolamins are characterized by the presence of repeated sequences. These are rich in proline and glutamine and appear to be based on similar motifs in the S-rich and S-poor groups. However, whereas these repeats are present only in the N-terminal parts of the S-rich prolamins, they account for almost the whole protein in the S-poor group. The repetitive sequences present in the HMW subunits are located in the center of the proteins, and are based on several motifs which are not related to those present in the S-rich and S-poor prolamins (Figure 13.8) [21, 22].

13.2.1 Genetics and Polymorphism

Genes coding these proteins are located on the short arms of groups 1 and 6 chromosomes (Figure 13.9). At least 50 different genes control the synthesis of gliadins. They are tightly linked genes located at three homologous loci of the group 1 chromosome, *Gli-A1*, *Gli-B1*, and *Gli-D1*, and the group 6 chromosomes, *Gli-A2*, *Gli-B2*, and *Gli-D2*. *Gli-1* genes encode all the ω- and most of the γ-gliadins and some of the β-gliadins, while genes of the 6 chromosomes *Gli-2* genes encode all the α-, most of the β-, and some of the γ-gliadins. Each cluster encodes a number of polypeptides that are inherited as a Mendelian character and multiple allelism has been established in both *Gli-1* and *Gli-2* loci. Of the three main wheat gliadin families, the ω-gliadins differ from the others because they generally have no cysteine or methionine. They are therefore defined as sulfur-poor prolamins, together with the ω-secalins of rye and the C-hordeins of barley [23].

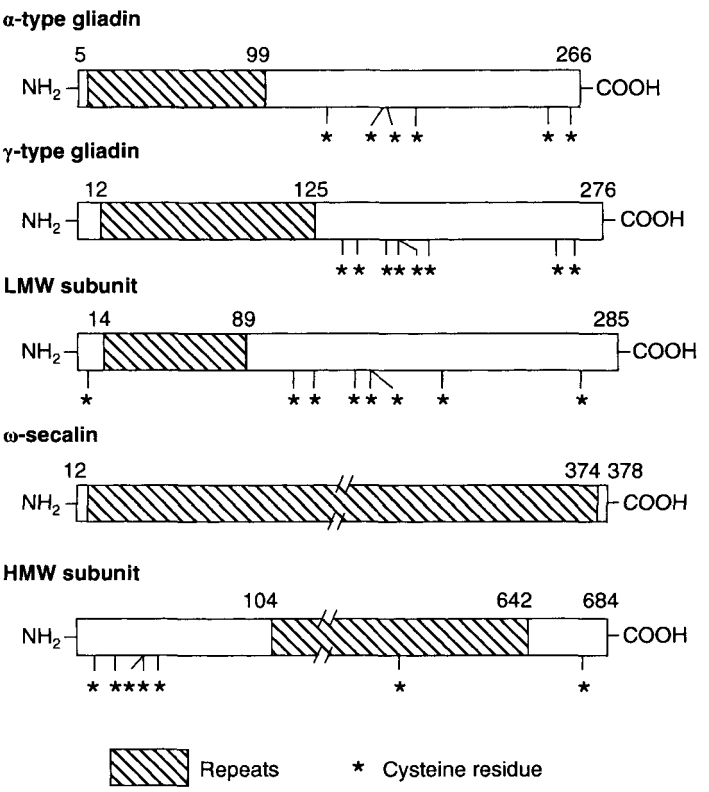

Figure 13.7 Schematic sequences of a typical α-gliadin, γ-gliadin, LMW subunit of wheat, and a ω-secalin of rye. The repeated sequences present in the gliadins, LMW subunits and ω-secalin are based on related motifs and are rich in proline and glutamine. The repeated sequences in the HMW subunits are not related to those present in the other proteins [12, 21].

On the basis of the N-terminal sequences, three different types of ω-gliadins have been observed in wheat and in related proteins such as C-hordeins and ω-secalins. These sequences are named ARQ-, KEL-, XRQ-, or RQ- and SRL-types on the basis of the first three amino acids of their N-terminal sequences : the ω-1 type begins with KEL, the ω-2 type with ARQ, ARE, XRQ, or RQ, and ω-5 type with SRL; the ω-5 type corresponds to the 1B ω-gliadins, whereas the 1A and 1D ω-gliadins include both the ω-1 and ω-2 types [24]. According to Kasarda *et al.* [24], the ARQ-type is thought to be the ancestral sequence type. The KEL-type differs from the ARQ-type in its lack of the eight residues and also with respect to a highly conserved sequence in the first 10 positions. The third type of ω-gliadin N-terminal sequence is the SRL-type, which is characteristic of ω-gliadins encoded by chromosome 1B [25]. The primary sequences of C-hordeins and ω-gliadins from the 1D chromosome are based on an octapeptide repeat motif made almost entirely from glutamine and proline residues in the sequence PQQPFPQQ. However, ω-gliadins encoded at chromosome 1B are characterized by the different internal peptides rich in glutamine such as QQXP, QQQXP, and QQQQXP, where X is F, I, or L in order of predominance [25].

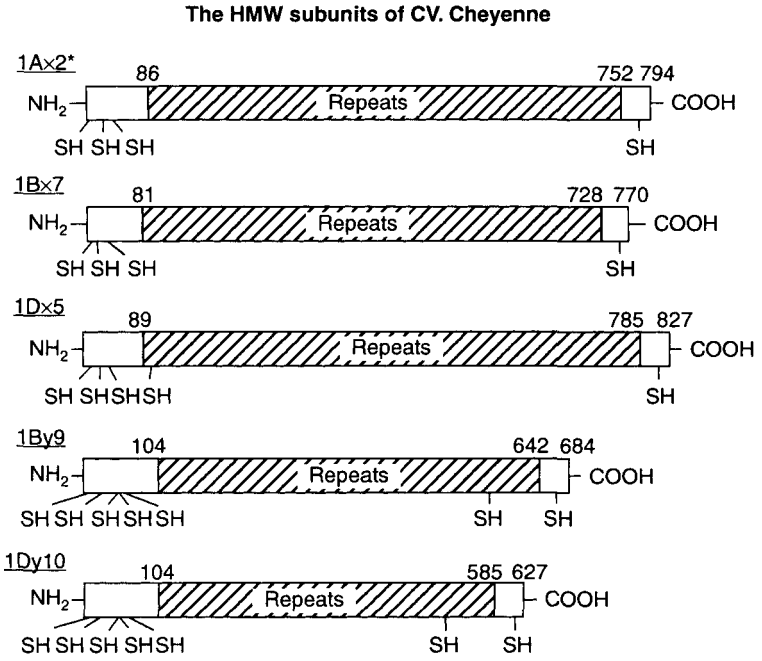

Figure 13.8 Schematic sequences of the five HMW subunits presented in the good quality bread wheat cv. Cheyenne. Cysteine residues are indicated by SH. Each group 1 chromosome contains two genes encoding a high M_r y-type subunit, but only five genes are expressed in cv. Cheyenne to give subunits 1Ax2*, 1Bx7, 1By9, 1Dx5 and 1Dy10 [21, 22].

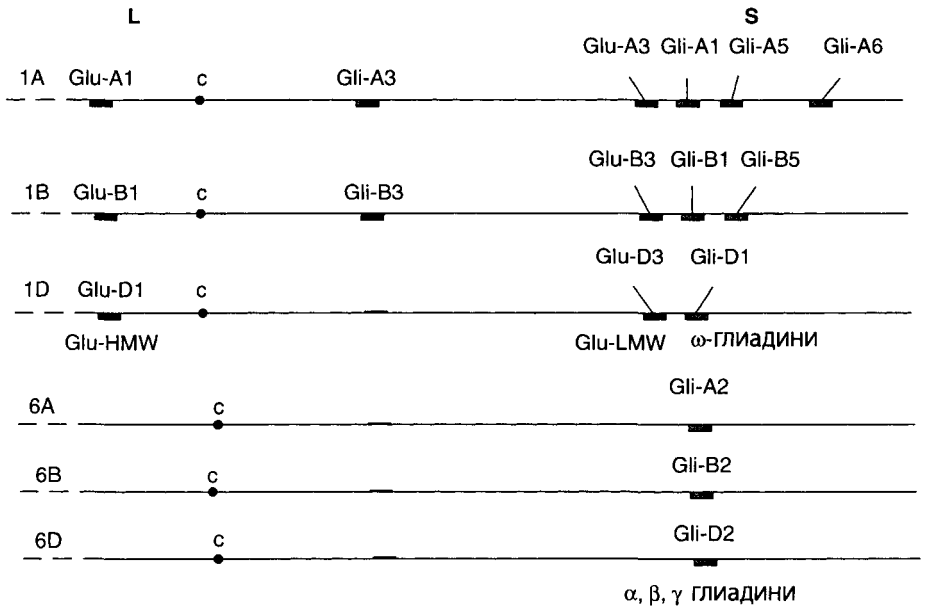

Figure 13.9 Chromosomal location of the Glu- and Gli- loci in T. aestivum L.

The study of gliadin related C-hordeins served as a model for understanding the structure of the other S-poor prolamins [26]. The ω-gliadins are homologous to rye ω-secalins and barley C-hordeins.

13.3 Glutenins

Glutenins play an important part in the breadmaking process, adding strength and elasticity to the dough.

Glutenins contain different polypeptides connected by intermolecular disulphide bonds; the polypeptides are called subunits and are subdivided into high-molecular-weight (HMW) and low-molecular-weight (LMW), according to their molecular weight when separated on sodium dodecyl sulphate polyacrilamide gels.

The glutenin fraction is formed of a mixture of polymers, high-molecular weight glutenin subunits (HMW-GS), and low-molecular-weight glutenin subunits (LMW-GS). The large glutenin polymers are stabilised by inter-chain disulphide bonds [8]. The HMW-GS have molecular weight ranging from 80–200 kDa (Figure 13.10) [13] and the LMW-GS weights are 30–51 000.

The HMW-GS account for about 5–10% of the total protein [27]. The LMW-GS most closely resemble γ-gliadins in sequence and comprise about 20–30% of the total protein [28]. Three to six HMW-GS [29] and 15–20 different LMW-GS proteins are recognised in 1 and 2D gels of hexaploid wheat [30].

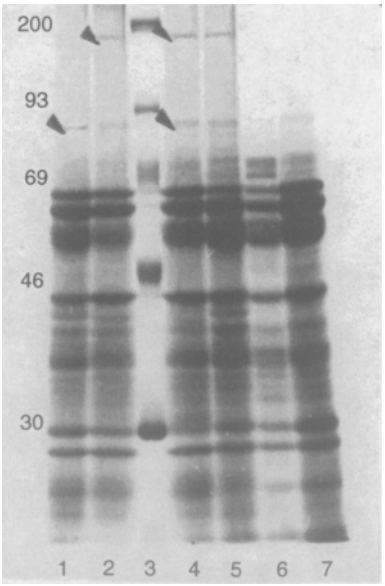

Figure 13.10 SDS-PAGE of seed storage proteins of T. aestivum L., cv S. ranozreika 2 and their mutant forms. Lane 1- control of T. aestivum, 2, 4, 5, 6, and 7 are lanes of mutant forms type sphaerococcum. The HMW-glutenin subunits are with high weight (more than 160,000), and well separated from other prolamins [13].

The HMW-GS consist of nonrepetitive domains of 88–104 and 42 residues at the N- and C-termini, respectively, separated by a longer repetitive domain (481–690 residues). Variation in the repetitive domain is responsible for most of the variation in the size of the whole protein, and it is based on random and interspersed repeats of hexapeptide and nonapeptide motifs, with tripeptides also present in x-type subunits only. Structure prediction indicated that the N- and C-terminal domains are predominantly α-helical, while the repetitive domains are rich in β-turns. Many partial and full-length sequences of HMW-GS and LMW-GS have been determined [31]. Despite the high degree of similarity in general structures and amino acid sequences of x- and y-type HMW-GS, some important differences are potentially critical for the structure and functionality of glutenin polymers [22]. The three differences are in: 1) molecular weight (x-type are bigger than y-type) due to a difference in length of the central repetitive domain; 2) the repeat structures in central domain; 3) the number and distribution of cysteine residues.

13.3.1 Gluten Polymer Structure

Several models for the structure of wheat gluten polymer have been proposed. According to earlier models, glutenin has only intra-chain disulphide bonds. The intra- chain disulphide bonds were thought to force glutenin molecules into specific conformations that facilitated interaction of adjacent glutenin molecules through non-covalent bonds, thereby causing aggregation [32]. Ewart (1979) [33] proposed an alternative model in which the adjacent polypeptide chains of glutenins were thought to consist of linear polymers and two adjacent chains were connected to each other with one disulphide bond. In this model, the rheological properties of dough are dependent on the presence of rheologically active disulphide bonds and thiol groups as well as on secondary forces in the concatenations.

Another model was proposed by [34] in which the functional glutenin complexes contained both inter- and intra-chain disulphide bonds. On the basis of results from SDS-PAGE, they proposed an aggregate of two types of glutenin complexes, I and II. In their model, glutenin I comprised subunits of molecular weight 6.8 x 104 and lower, held together through hydrogen bonds and hydrophobic interactions; glutenin II comprised crosslinked subunits of molecular weights above 6.8 x 104, linked by inter- chain disulphide bonds.

More recent studies have shown that gluten protein polymers have a wide range of size distribution, ranging from dimers to polymers with molecular weights up to millions (possibly exceeding 1 x 107) [12]. The proteins with the highest molecular weight are reported to have the strongest correlation with strong dough properties [35]. A certain amount of these polymers remain unextractable in various extracting systems (acetic acid solution or SDS phosphate buffer). The 10% UPP (percentage unextractable polymeric protein in total polymeric protein) is often used as a measurement of the amount and size distribution of the polymeric protein [36]. High % UPP values are related to a greater proportion of glutenin that is insoluble in SDS, and for that reason are

thought to be of the highest molecular weight [37]. Thus, wheat with a greater percentage of UPP are expected to have a greater dough resistance (elasticity) and a longer mixing requirement than those with a greater proportion of extractable polymeric protein [38].

13.3.2 Polymeric Proteins

Because of their functional importance, the gluten proteins have been studied in detail using genetical, molecular, biochemical, and biophysical approaches. This has revealed details of the numbers and locations of their controlling genes loci, their amino acid sequences, their conformations, and their relationship to various functional properties [39].

With the molecular weights of glutenin polymers reaching over twenty million daltons, based on gel filtration [40] and flow field-flow fractionation (FFF) studies, glutein proteins are among the largest protein molecules in nature [12]. These proteins are heterogenous mixtures of polymers formed by disulfide-bonded linkages of polypeptides that can be classified in four groups according to their electrophoretic mobility in SDS-PAGE after reduction of S-S bonds (the A-, B-, C-, and D-regions of electrophoretic mobility) (Figure 13.11) [41].

The A-group (with an apparent molecular weight range of 80,000–120,000 Da) corresponds to the HMW-GS [42]. The B-group (42,000–51,000 Da), and C-group (30,000–40,000 Da) are LMW-GS distantly related to γ- and α-gliadins [42, 43]. Finally the D-group, also belonging to the LMW-GS group, is highly

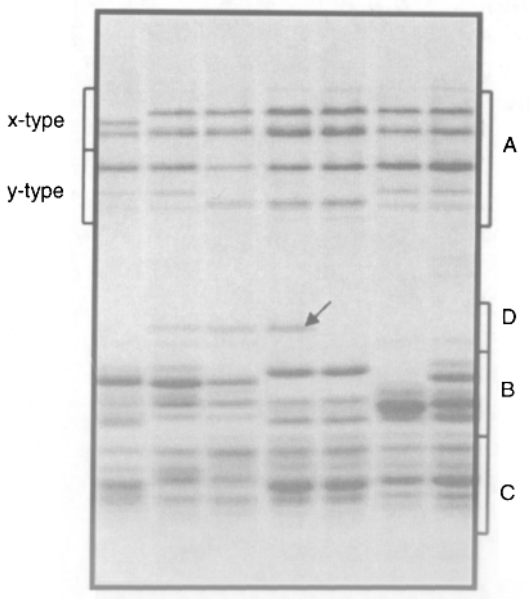

Figure 13.11 SDS-PAGE of polymeric protein (after reduction to subunits). Group A: HMW glutenin subunits showing x- and y-type subunits. Group B-, C-, D-: LMW glutenin subunits. Arrow indicates subunit D [41].

acidic and related to ω-gliadins [44] and by reversed-phase HPLC [45]. These are valuable techniques that offer excellent resolution, automation, quantitation, and computerization. Based on separation by differences in charge and hydrophobicity, they can be used alone or complementary to other separation methods (mainly SDS-PAGE).

A wide range of approaches has been used to provide better purification of the glutenin fraction. After Osborne's studies, Jones *et al.* (1959) [46] tested two different methods (ethanol 70%, pH fractionation) for precipitating glutenin from gluten.

The pioneer studies of Bietz and Wall (1972) showed that two types of subunits were present, the low molecular weight (LMH-SG-10,000-70,000Da) and the high molecular glutenin subunits (HMW-SG-≈80,000-130,000Da) [47].

The HMW-CS (80,000-130,000Da), which represent approximately 0.5 % of the total seed dry weight, have been studied extensively because of their effect on elasticity, and hence the breadmaking quality of wheat dough. Bread wheat is a hexaploid species comprised of three different but related genomes, A, B, and D. Using nulissomic-tetrasomic, nullisomic-trisomic, and ditelocentric lines of Chinese Spring, Bietz *et al.* (1975) [48] showed that HMW-GS were controlled by genes at the long arms of chromosomes 1A, 1B, and 1D . The HMW-GS genes (*Glu-1*) are located on the long arms of the homologous chromosomes 1A, 1B, and 1D (Figure 13.9). These loci are named *Glu-1A*, *Glu-1B*, and *Glu-1D*. Each locus includes two genes linked together encoding two different types of HMW-GS, x- and y-type subunits based on their electrophoretic mobility and isoelectric points [22, 49]. The x-type subunits generally have a slower electrophoretic mobility in SDS-PAGE and high molecular weight than y-type subunits. Tightly linked pairs of one x-type and one y-type gene are present at each locus on the 1A, 1B, and 1D chromosomes of hexaploid wheat. The x- and y-type genes appear to have been formed by a duplication of a single ancestral gene early on during wheat evolution [50]. They have been maintained separately as two distinct genes in each HMW-GS locus, rendering x- or y-types of different wheat genomes more homologous to each other than to x- and y-types encoded by the same genome [21]. This is in contrast to other groups of wheat storage proteins, which comprise multigene families and apparently evolved by unequal crossing over [51, 52].

The x- and y-type HMW-GS share a very similar structure (about 80% amino acid sequence similarity); a large central domain composed of repeating amino acid sequences rich in glutamine and proline, flanked by N- and C-terminal domains made up of non-repetitive sequences that contain highly conserved cystine residues (Figure 13.12) [53].

Nevertheless, x- and y-type subunits differ in several important features. X-types usually have three cysteines in the N-terminal domain, while y-types have five; both types have one cysteine in the C-terminal domain, but y-types have an additional cysteine within the repeat region in close proximity to the C-terminal domain (Figure 13.12). In addition, there some differences between the two types in the composition and order of repeats within the central domain [23]. Allelic variation in HMW-GS size is caused by deletions or duplications

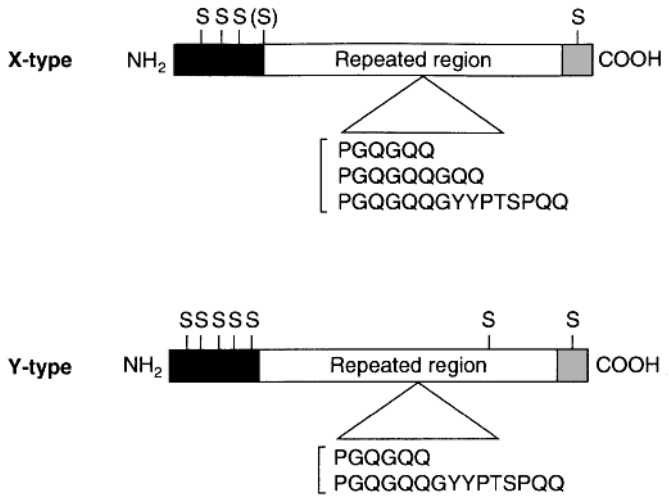

Figure 13.12 Structural aspects of the x- and y-type HMW-GS gene. The two HMW-GS types have a similar structure: a central repetitive domain comprising repeats of six, nine and 15 amino acids and smaller terminal domains containing most of the cysteine residues (disulfide bonds are indicated by 'S': The x-type HMW-GSs apparently have one intramolecular disulfide bond within the N-terminus, and the y-type is likely to have two such bonds, thus reducing the cysteines available for forming intermolecular bonds necessary for producing the glutenin macro-polymer [53].

within the repetitive domain, caused by recombination events among the repeated DNA sequences.

Six HMW-GS genes are present in each cultivar of hexaploid wheat, but because of gene silencing only three, four, or five subunits are synthesised in different cultivars. The HMW-GS 1Bx, 1Dx, and 1Dy are present in all cultivars, 1By and 1Ax are present in some cultivars, but no cultivars contain 1Ay because the gene is always silent. Similarly, the tetraploid durum wheats, with the A and B genomes, contain four HMW-GS genes, of which no more than three are active. Electrophoretic studies have revealed appreciable polymorphism in the number and mobility of HMW-GS in both bread wheats – 6x and 4x [54]. Consequently, the *Glu-1* loci present multiple allelism. Payne and Lawrence (1983) [55] summarized the range of the alleles at the *Glu-1* loci as three allelic forms at the *Glu-1A*, 11 alleles at the *Glu-1B*, and six alleles at the *Glu-1D*. The new allelic forms at the *Glu-1A* were also described in T. Durum [13]. *Glu-A1* locus is less polymorphic, than *Glu-B1* and *Glu-D1 loci*. Ikeda *et al.* (2008) [56] described eight allelic forms at the *Glu-A3*, 22 alleles at the *Glu-B3* and nine alleles at the *Glu-D3*. Wild species such as Aegilops, T. tauschii, and T. monococcum have also shown extensive polymorphism of HMW-GS [57–59].

13.3.3 Structure

Little was known about the structure of these proteins until 20 years ago. Then, boosted by new technology developments such as molecular cloning, it became possible to isolate cloned cDNA and genes for all the major groups of gluten

proteins [61–63]. This has allowed for the complete amino acid sequences of the proteins encoded by these DNA to be deduced, providing a basis for modeling and biophysical studies. Such molecular and biophysical studies have been combined to give a detailed picture of HMW-GS structure. Contrary to the central repetitive domain, where a regular spiral structure has been proposed, the structure for both terminal regions is α-helical [63].

PCR is a fast and reliable alternative to more conventional methods for the study of wheat protein genes. D'Ovidio *et al.* (1995) [64] reported specific amplifications of the complete coding region of all six HMW-GS genes present in hexaploid wheat by means of PCR. This permitted ready analysis of the genetic polymorphism of HMW-GS genes as well as the isolation of new allelic variants. Also they proposed that the length variation observed for the HMW-GS encoded at the *Glu-1* locus is mainly due to variation in the length of the central repetitive domain [64]. This has been confirmed by PCR analysis using primers specific for the N-terminal, C-terminal, and repetitive regions of HMW-GS genes at the *Glu-D1* locus in hexaploid wheats.

13.3.4 Relationship to Wheat Quality

The gluten proteins are clearly of greater importance in determining the functional properties of wheat doughs. Three aspects of gluten protein structure are of interest: The structures of the individual proteins, their interactions with each other, and their interactions with other dough components (starch, lipids). There is no doubt that the repetitive domains and the structures that they adopt are of great importance in determining the properties of gluten. It has been well documented that the *Glu-D1* encoded HMW-GS pair 1Dx5 + 1Dy10 [65] is associated with greater dough strength, while the allelic variant pair 1Dx2 + 1Dy12 is associated with lesser strength. Other allelic variant pairs had similar results: *Glu-B1* subunits 1Bx17 + 1Dy18 (strong) versus subunits 20x + 20y (weak). The viscosity (extensibility) of gluten almost certainly results primarily from strong hydrogen bonding and hydrophobic interactions between the repetitive domains of the gluten proteins, notably the monomeric gliadins.

Whereas gluten is mainly determined by non-covalent interactions, particularly between the gliadins, elasticity is associated with the covalently glutenin polymers and with the HMW subunits. Although the HMW subunits only account for about 20% of the total glutenin fraction (and 6–10% of whole gluten), two lines of evidence indicate that they are associated with high elasticity and good breadmaking quality. Also, it is well know that allelic variation in their number and composition is strongly correlated with variation in breadmaking quality [50]. Thus, all cultivars contain two subunits encoded by chromosome 1D and one encoded by chromosome 1B, while additional 1B-encoded and/or chromosome 1A-encoded subunits are present in some cultivars only. Variation in bread-making quality is correlated with the absence or presence of a subunit encoded by chromosome 1A, and with allelic variation in the two subunits encoded by chromosome 1D.

Little is known about the precise organisation of the HMW and LMW subunits in glutenin polymers, but most researchers consider that the HMW subunits come from a disulphide-bonded network, with the LMW subunits acting as branches, possibly also providing some cross-links. The formation of such a network by the HMW subunits would be facilitated by the distribution of cysteine residues, which are predominantly located in the N-terminal (3 or 5 cysteines) and C-terminal (1 cysteine) domains (Figure 13.12). It would be possible for the HMW subunits to form polymers via head-to-tail disulphide bonds, with some cross-links (either directly or via LMW subunits) and branches. In addition, one or two intra-chain disulphide bonds could be formed within the N-terminal domains. The number and distribution of cross-links within these glutenin polymers would undoubtedly contribute to their elastic properties.

A second property of the HMW subunits which could relate to elasticity are the properties of the β-turn rich spiral supersecondary structure formed by their repetitive domains. It has been suggested that this structure is intrinsically elastic and contributes directly to the elasticity of the glutenin polymers [66].

The HMW subunits appear to have quantitative effects on breadmaking quality. Although all bread wheats have six HMW-GS genes, only three, four, or five of these are expressed. These differences in gene expression are associated with quantitative effects on the total amount of HMW protein, each gene accounting on average for about 2% of the gluten proteins. This may account for the higher breadmaking quality of cultivars containing subunits encoded by chromosome 1A [50]. However, variation in quality is also associated with allelic variation in expressed HMW subunits. In such a case the effect on quality could result from differences in HMW subunit structure, either in their ability to form cross-links (for example, one quality associated with subunits encoded by chromosome 1D contains an additional cysteine residue compared with an allelic subunit associated with poor quality), or in the intrinsic elasticity of the repetitive domain [22].

In case of bread wheat, increases in gluten elasticity could be obtained by inserting additional genes for HMW-GS to increase the total amount of HMW subunit protein. In addition, these genes could possibly be mutated to give more subtle differences, due to effects on the cross-linking or other properties of the glutenin polymers.

It must be point out that wheat gluten is present as a network in dough, where it is intimately associated with other dough components. These interactions are still not completely understood, but there are reported correlations of breadmaking quality with polar lipid content, so lipids are potentially of great importance in modulating the functionality of the gluten proteins [39].

13.4 LMW-GS

Low-molecular-weight glutenin subunit (LMW-GS) composition in common wheat is one of the critical determinants of gluten properties. However, the nomenclature of *Glu-3* encoding LMW-GS has not been consistent among

laboratories, due to the complexity of the LMW-GS and the distinct separation methods used by different researchers. Ikeda *et al.* (2008), [56] using 1D SDS-PAGE and 2D analysis, investigated more than 103 cultivars of T. aestivum L., from Argentina, China, France, Japan, and Mexico in order to find out about various *Glu-A3*, *Glu-B3*, and *Glu-D3* alliles. It is very important to unify the various *Glu-3* allelic nomenclature systems in use.

The LMW-GS are controlled by genes at the *Glu-A3*, *Glu-B3*, and *Glu-D3* loci on the short arms of chromosome 1AS, 1BS, and 1DS, respectively (Figure 13.9). Chromosome 1A encodes relatively few LMH-GS. On the other hand, there is extensive polymorphism for LMW-GS encoded by chromosome 1B. There is also evidence that some genes *Gli-A2*, *Gli-B2*, and *Gli-D2* of the LMW-GS are controlled by genes of the short arms of chromosome 6 (6A, 6B, and 6D) [30, 38]. A Close linkage also exists between the *Glu-3* loci encoding LMW-GS and the *Gli-A1*, *Gli-A5*, *Gli-A6* loci [67, 68] *Glu-B3*. The *Gli-1* multigene loci encode γ- and ω-gliadins and some β-gliadins at the distal ends of the short arms of chromosome 1A, 1B, and 1D.

The LMW subunits are clearly a key target for attempts to improve durum wheat quality by genetic engineering. Also, it is important to point out that durum wheat, which indicated high gluten strength with good quality, is related to the content of S-rich prolamins rather than to the HMW subunits. Durum wheat cultivars can be divided into two groups characterized by the absence or presence of γ-gliadins called bands 42 and 45 respectively [69]. Each of these bands is, in fact, a marker for the complex *Gli-B1* locus, which encodes a mixture of γ-gliadins, ω-gliadins and LMW subunits of glutenin, and it is probable that specific blocks of LMW subunits associated with gliadins 42 and 45 are responsible for the associated weak and strong dough respectively [69]. Pogna *et al.* (1990) and Ruiz and Carrillo (1995) [68, 71] demonstrated that LMW glutenin subunits were responsible for the quantitative differences in quality.

Recently, two new LMG-GS with molecular weights of ≈30-31,000 Da (*Glu-D4* locus) and 32,000 Da (*Glu-D5* locus) were reported [72]. These genes are located on chromosome 1D and 7D, respectively, although their exact location within the chromosome has not been established.

The LMW-GS (B-, C-, and D-subunits) represent about one-third of the total seed protein and ≈60% of total glutenins [73]. Despite their abundance, they have received less research attention than HMW subunits. This has been mainly due to the difficulty in identifying them in one dimensional SDS-PAGE gels. The resolution of the problem, which was principally due to overlapping between LMW-GS and gliadins, was resolved by Singh and Shepherd (1988) [67] who developed a two-step SDS-PAGE method. Advances in the characterization of LMW-GS have been enhanced by the production of wheat-rye translocation lines (single, double, and triple), permitting the simplification of the electrophoretic pattern for closer study of the alleles of LMW-GS. RP-HPLC has also proved useful for the study of LMW-GS, showing that these proteins have higher hydrophobic surfaces than those from HMW-GS and are comparable with the hydrophobic surfaces of gliadins. Recent improvements in capillary electrophoresis (Bean and Lookhart 2000) [74], allow clear characterization of all glutenin subunits.

13.4.1 Structure

Several DNA sequences have been reported for LMW-GS genes [75]. The secondary structures of LMW-GS, except for the D-subunits, have an overall similarity with the structure of the S-rich gliadins [43, 64] (Figure 13.6). Most polypeptides consist of 250–300 residues. They possess a two domain structure, although further divisions within these have been reported [41]. The N-terminal repetitive domains are rich in β-turns, possibly forming a regular structure, while the short non-repetitive domains are rich in α-helix and appear to be more compact [76]. Repeated sequences account for ≈30-50 mol % of these proteins, in contrast to the more extensive repeats in the S-poor gliadins and HMW-GS. These N proteins have a cysteine residue within the N-terminal domain [30], which is unlikely to form intramolecular disulfide bonds with cysteine residues in the C-terminal domain because of the rigidity imposed by the repetitive sequence. In addition, LMW-GS have seven cysteine residues in their C-terminal domain, at least one of which is unpaired, thus available for intermolecular bonding.

The electrophoretic mobility and N-terminal sequences of the D-subunits are very similar to the S-poor ω-gliadins [77], and it looks like there are mutant forms of ω-gliadins in which a single cysteine residue allows cross-linking into the glutenin polymer. It has been proved recently that only one cysteine was involved in the structure of D-subunits, allowing them to act as chain terminators [77].

13.4.2 Molecular Characterization of LMW-GS Genes

The LMW-GS genes, like all other prolamin genes, do not have introns in their sequences [61]. The core technology of proteomics is 2D electrophoresis. At present, there is no other technique that is capable of simultaneously resolving thousands of proteins in a separation procedure. Two dimensional electrophoresis (2D electrophoresis) is a powerful and widely used method for the analysis of complex protein mixtures extracted from cells, tissues, or other biological samples. This technique sorts proteins according to two independent properties in two discrete steps: the first-dimension step, isoelectric focusing (IEF), separates proteins according to their isoelectric points (pI); the second-dimension step, SDS-poliacrilamide gel electrophoresis (SDS-PAGE) separates proteins according to their molecular weights. Each spot on the resulting two-dimensional array corresponds to a single protein species in the sample (Figure 13.13) [78].

Traditionally, 2D separations were performed by combining two types of gel electrophoresis, IEF and SDS-PAGE [79] separating first by pI then by size. More than 1000 components of wheat proteins have been resolved using a combination of isoelectric focusing (IEF) and sodium dodecyl sulfate PAGE (SDS-PAGE). The prolamin storage proteins of barley, wheat, and rye account for about half of the total grain protein. In barley (Hordeum vulgare L), the major prolamin fraction is composed of hordeins, which are further divided into several heterogenous groups, namely D (high molecular weight), C (sulphur-poor), B and γ (sulphur-rich) hordein peptides.

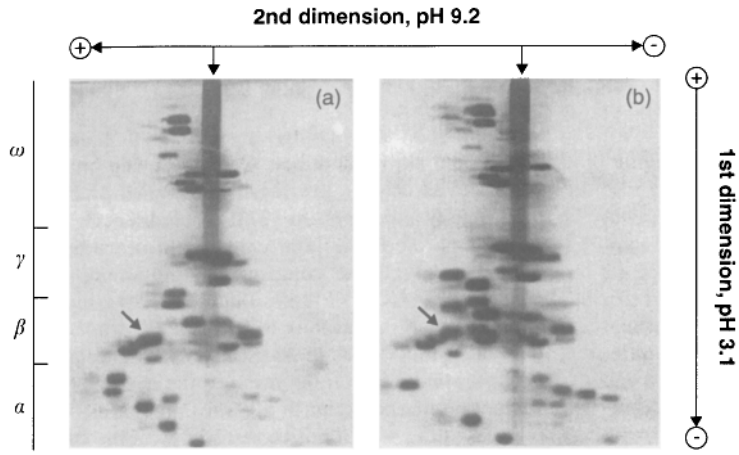

Figure 13.13 Two-dimensional separation of gliadin components extracted from nullisomic 6B-tetrasomic 6D, a. and ditelocentric 6DL of 'Chinese Spring' b. Component indicated was found to be controlled by the chromosome 6D analyzing the substitution lines 6D (6A) and 6D (6B) of 'Chinese Spring' in 'Langdon' [78].

The S-poor prolamins comprise the ω-gliadins of wheat, the ω-secalins of rye, and C hordeins of barley. The S-rich prolamins are the major group of prolamins present in wheat, barley and rye, accounting for approximately 80% of the total fractions. They comprise the α-gliadins, the γ-gliadins, and LMW glutenin subunits of wheat, the gamma and B hordeins of barley, and the 40 kDa gamma secalins of rye. The HMW subunits of wheat glutenin play an important role in determining the breadmaking quality of wheat. HMW prolamins are also present in barley and rye, where they are called D hordein and HMW secalins, respectively.

Neverthless, all hordein proteins share certain biochemical and physical characteristics. For example, they are insoluble in water or dilute salt solutions, soluble in alcohol-water mixtures, and have high glutamine and proline content. Numerous genes for prolamin proteins have been isolated from a variety of cereals, including those encoding B, C, D, and γ hordein. From a detailed sequence analysis, it was proposed the sulphur-rich and sulphur-poor hordeins are derived from the same ancestor gene [52]. Genetically, hordein genes all map to chromosome 5 (1H), with the D hordein gene on the long arm and other hordein protein genes on the short arm. Moreover, barley prolamin protein genes are present as multiple copies in the genome with the exception of the *Hor 3* locus encoding the D hordein gene [80].

Barley and wheat genomes are closely related with respect to their evolutionary origin. While barley (H. vulgare L) has a true diploid genome, bread wheat (T. aestivum L) is a hexaploid species consisting of three homologous genomes (A, B, and D), with each genome having seven mostly collinear chromosomes. Comparative studies using RFLP markers have revealed that the linkage groups of wheat and barley genomes are remarkably conserved [81]. The chromosomal regions carrying the hordein genes on chromosome 5 (1H)

are likely orthologous to the regions on the group 1 chromosome of each wheat genome. The corresponding region in the wheat genomes contains similar seed storage protein genes to those found in barley. On the long arms of group 1 chromosome are located genes encoding the wheat high molecular weight (HMW) glutenin subunits that are homologous to D hordein. The S-rich γ-gliadins and low molecular weight (LMW) glutenins, and S-poor ω-gliadins located on the short arms of group 1 chromosomes are the counterparts of the B, C, and, γ hordeins.

13.5 MALDI/MS: A New Technique Used to Analyze the Proteins in Plants

In recent years a new technique, MALDI/MS, was used to analyze proteins in plants. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI/MS) was used to analyze the protein composition in several common and durum wheat varieties. Mass spectra were obtained directly from crude and partially purified wheat gliadin and reduced glutenin subunit fractions. Mass spectra of the gliadins and the low molecular weight (LMW) glutenin subunits show a complex pattern of proteins in the 30–40 kDa range. The observed gliadin patterns may be suitable for differentiation between wheat varieties, but the complexity of the mass spectra precludes the use of MALDI/MS as a stand-alone technique for the identification of most individual gliadin components. The mass spectra of the high molecular weight (HMW) glutenin subunits are much simpler, and the complete HMW subunit profile can be determined directly from a single mass spectrum. This may prove particularly useful in wheat breeding programs for rapid identification of lines containing subunits associated with superior quality. The correspondence between previously identified HMW subunits and the mass spectral peaks was established with MALDI measurements of HPLC-separated subunits. Delayed extraction proved effective in improving the mass resolution for the monomeric gliadins and LMW glutenin subunit fractions, with masses less than 40 kDa. However, it provided little improvement for the HMW glutenin subunits which have masses of ~80 kDa [82].

13.6 Albumins and Globulins

Storage proteins which are soluble in water and in salt solutions are albumins and globulins. Many of them are enzymes or enzyme inhibitors. Their apparent molecular weight from SDS-PAGE are lower than the gliadins (<30,000 Da). Genes for the major albumins and globulins of wheat have been assigned to chromosome groups 3, 4, 5, 6, and 7 [83]. A major component of the albumins of lower molecular weight (14,000-16,000 Da) was encoded by the short arm of chromosome 3D, as suggested by its absence in water extracts of aneuploid strains nullisomic-3D, tetrasomic-3B, and ditelosomic-3DL of Chinese Spring.

Also they differ in amino acid composition from the gluten proteins, possessing lower amounts of glutamic acid and more lysine. Unfortunately, because they are present in the wheat endosperm in minor proportions, their presence it is not enough to overcome the lack of lysine in wheat. Analysis of aneuploid stocks of the wheat variety Chinese Spring has revealed that the HMW albumins of 69, 63, 60, and 45 kDa are controlled by genes on the chromosome arms 4DL, 4AL, 5AL and 5DL respectively.

13.7 Wheat Gluten and Dietary Intolerance

Gluten is among the most important food components accounting for hypersensitivity reactions in children. Adverse reactions to gluten protein include food allergy, food dependent exercise-induced asthma, anaphylaxis, and coeliac disease. Sensitization to gluten by ingestion can lead to food allergy symptoms, whereas sensitization by inhalation causes baker's asthma and rhinitis. For example, although the mechanism is not fully understood, wheat fast omega-5 gliadin and alpha-gliadin has been shown to be a major allergen in children with immediate allergy to ingested wheat. Other researchers have shown that alpha-gliadin and gamma-gliadin in addition to LMW glutenin are the allergens for patients with wheat allergies.

Baker's asthma is a frequent allergy in the baking industry. In Germany, approximately 1,800 bakers annually claim compensation for baker's asthma. In Japan, in recent years the number of patients suffering from baker's asthma caused by bread wheat has been increasing. Non-IgE immune reactions to gluten may result in celiac disease, which is widespread, occurring in 0.5-1% of the population. Celiac disease is traditionally associated with European countries, particularly Scandinavia, also North Africa, the Middle East, and South Asia. The prevalence of celiac disease (CD) among school children from India is not rare in wheat-eating areas of North India [84]. CD may occur at any age. In infants, symptoms appear only a few months after the introduction of foods containing gluten (6–2 months). In adulthood the onset is usually between 30–40 years. It should be noted that CD affects twice as many females as males.

Many researchers have been trying to understand the mechanism of CD and to reduce allergenicity of the protein by modifying the allergen structure in such a way that the allergenic epitopes are no longer recognized by the immune system. Until now, the technological approach to decrease allergenicity has largely been empirical. The main reason for this is a lack of detailed knowledge about the integral allergen and epitope structure and their genetic background.

The extensive research and education of consumers is needed to fully understand the value of gluten protein in terms of its possible or particular nutritional health benefits or defects. However, gluten does have economic benefits over and above the more expensive milk or soy protein products, and its functional properties, which other products cannot duplicate, give it a unique place among the various protein products.

13.8 Conclusion

A great deal of research attention has been focused on the study of wheat gluten proteins in the past three decades. Of all the cereal grains, wheat is unique because wheat flour alone has the ability to form dough that exhibits the rheological properties required for the production of leavened bread and many other foods. The unique properties of the wheat grain residue are primarily in the gluten forming storage proteins of its endosperm. The protein that makes up gluten is a complex mixture of proteins, containing many – probably several hundred – polypeptides, about half of the protein being monomeric (gliadins) that comprise approximately 50% of total gluten proteins, and the remainder being disulfide cross-linked polypeptides that form the polymeric glutenin fraction, whose size ranges up into the tens of millions of Daltons. The major groups of wheat prolamins are the high-molecular-weight (HMW) glutenins encoded by genes on the long arm of the group-1 homeologous chromosomes, the α-gliadins encoded on the groupe-6 chromosomes, the γ-and ω-gliadins, and the low-molecular weight (LMW) glutenins all encoded by genes located on the short arm of the group 1 homologous chromosomes. Given the unique properties of wheat gluten, it is not surprising that it has been the subject of intense scientific investigations during the last decade. From a nutritional point of view, protein is considered to be limited in the essential amino acids like lysine and tryptophan, and hence there is a need for using various molecular approaches such as recombinant DNA technology, protein sequence modification, and synthetic genes, to improve nutritional quality. The techniques of genomics and proteomics provide considerable opportunities in this area. By comparing profiles of gene expression and protein accumulation and pattern under different environmental conditions, it should be possible to reveal basic molecular mechanisms that are influenced by environment and affect productivity and quality. It should be noted that molecular mechanisms involved in wheat grain development are still poorly understood despite the importance of cereals as a major source of nutrition for human kind. Obviously much more work is required to resolve this question.

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14

Natural Rubber: Production, Properties and Applications

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Abstract

Though over 2000 different plant species produce natural rubber (NR), the main commercial source of the material is *Hevea brasiliensis*, the rubber tree. NR was introduced to Europe by Christopher Columbus by the late 15th century, but its organised production to meet the growing industrial demand is just over 100 years old. The technical basis for large-scale industrial use of rubber was laid first in 1839 when Goodyear discovered vulcanization, and then in 1888 when Dunlop reinvented the pneumatic tyre. Now about 10 million tonnes of NR is being produced in over 20 countries, mostly in tropical Asia and Africa. While the automotive tyre is the main area of application of NR, over 35000 different products are made from it, influencing the quality of human life in several ways. History, cultivation, global statistics, properties, manufacturing technology, commercial blends, chemical modification, and the green credentials of NR are discussed in this chapter.

Keywords: Natural rubber, *Hevea brasiliensis*, cultivation, modification, primary processing, manufacturing techniques, pneumatic tyre, non-tyre products, vulcanization, green commodity

14.1 Introduction

Natural rubber is one of the most wonderful gifts of nature with very unique properties. It is highly elastic, which makes it soft yet tough. It gently but firmly holds things together and absorbs bumps and shocks. The material is highly impermeable to air and does not conduct heat and electricity. These properties have made natural rubber almost indispensable, and several thousands of products are made from it, influencing the quality of human life in so many ways. From tiny rubber bands to giant earth mover tyres, the range of products made from natural rubber is very broad. So varied are its applications that products made from natural rubber can prevent the birth of a child and at the

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (403–436)

same time can be used to feed a new born. Natural rubber production provides a livelihood to over 10 million small farmers, mostly in the developing countries of Asia and Africa. Because of its plant origin which requires a minimum amount of energy other than that from sunlight for production, and its unique cultivation practices involving a minimum use of agricultural inputs, natural rubber is widely recognized as a 'green commodity'.

The word 'rubber' was first coined in 1770 by the British chemist Joseph Priestly to describe the material that was sold by an artist's shop in London for erasing or 'rubbing out' pencil marks. The other names for rubber include 'India rubber' (the material obtained from the West Indies), 'Para rubber' (rubber sourced from the state of Para in Brazil), 'Hevea rubber' (rubber from *Hevea brasiliensis*), 'Caoutchouc' (from Spanish, meaning wood that weeps), 'Seringa' (product of the 'syringe tree'), and 'Guayule' (rubber from guayule plant found in the semi-arid regions of Mexico). At present, the word 'rubber' is extended in common usage to include an ever growing range of synthetic materials with similar properties. However, even the synthetic chemical equivalent of natural rubber, isoprene rubber, does not compare well with the natural material in all its scientific and technical characteristics.

14.2 Rubber Yielding Plants

A detailed account of various rubber yielding plants is provided by George and Panikkar [1], and more recently by van Beilen and Poirier [2], Buranov and Elmuradov [3], and Pearson *et al.* [4]. Natural rubber is found to be present in over 2000 species of plants belonging to 311 genera of 79 families. Most rubber bearing plants contain latex, but in a few, small particles of rubber are scattered in the tissues. The majority of rubber bearing plants belong to a few plant families such as Euphorbeaceae, Moraceae, Apocynaceae, and Asteraceae. Over 99% of the total global production of natural rubber is from the Para rubber tree (*Hevea brasiliensis*), a member of the *Euphorbiaceae family*. The genus Hevea includes other species such as *Hevea benthamiana, Hevea camargoana, Hevea pauciflora, Hevea spruceana,* and *Hevea rigidifolia.* However, these less important species of Hevea have not attained commercial importance for rubber production.

Prior to the identification of *Hevea brasiliensis* as the most important commercial source of natural rubber, plants belonging to a wide spectrum of families had been explored for extraction of rubber. Among these, extractable quantities of rubber could be obtained from a few sources as given in Table 14.1.

14.3 History

Rubber is now so much a part of our daily life that we often take it for granted and tend to believe that it has been with us for a long time. However, the discovery and use of rubber is surprisingly very recent. A detailed history of the evolution of natural rubber as one of the most important industrial raw materials

Table 14.1 Minor plant sources of rubber.

Family	Botanical name	Country of origin	Type	Common name
Euphorbiaceae	*Manihot glaziovi*	Brazil	Tree	Ceara rubber
Moraceae	*Castilla elastica*	Central America	Tree	Castilla(Panama) rubber
	Ficus elastica	India, Burma	Tree	Assam rubber
Apocynaceae	*Funtamia elastica*	African coast	Tree	Lagos silk rubber
Asteraceae	*Parthenium argentatum*	North Mexico	Shrub	Guayule (Mexican) rubber
	Taraxacum kok-saghyz	Crimea	Shrub	Russian dandelion rubber
Asclepiadaceae	*Urceola elastica*	India	Tree	White Assam rubber

has been given by Loadman [5] in his book *Tears of the Tree* and by IRRDB [6] in the *Portrait of the Global Rubber Industry*. Although the bouncing balls made from certain plant exudates have been used by some Mesoamericans from as early as 1600 BC, the elastic material has been known to the civilized world only from the late 15th century, when the European explorers discovered the 'New World' in the American continents and brought with them the elastic material to Europe. Rubber products, mainly balls and shoes, had been arriving in Europe regularly from the 16th century. But it was the Frenchman François Fresneau who first identified turpentine as solvent for rubber and recognized the technological and commercial potential of natural rubber. Together with his friend Charles de la Condamine, a report on the characteristics and potential uses of this unique material, was presented to the *Academie Royale de Sciences* in Paris in 1765. La Condamine was the first to use the word 'latex', derived from the Spanish for milk, to describe the sap of the tree giving the latex. He was also the first to refer to rubber by the Native American name 'caoutchouc' meaning 'wood that weeps'. With the discovery of turpentine as solvent for rubber by Fresneau and later naphtha by James Syme, an Edinburgh medical student, many applications were developed for rubber which included water-proof fabrics, catheters, balloons, etc. The first rubber factory was established in Paris in 1803, followed by those in Austria in 1811, Germany in 1829, and Russia and the USA in 1830. The rapid economic progress in the USA in the 1830s saw the establishment of several rubber factories along the Pacific Coast. It was Charles Macintosh, a chemical manufacturer in Glasgow, who first used rubber solution in naphtha as a waterproofing layer between two fabric layers to develop the well-known 'Macintosh' waterproofing process in 1823, and a factory was opened in Manchester in 1824 for its production. Very close to the

development of waterproofing of fabrics, the discovery of mastication, a process of the mechanical working of rubber, was made by Hancock, who later came out with processes such as vulcanization, addition of fillers to rubber, compression moulding under heat, solution dipping, and latex thread technology.

By the mid-1830s, there was a flourishing rubber manufacturing industry in Britain and North America, but all the products were unvulcanized. The extreme temperature changes in America made the rubber products either sticky due to the excessive heat in summer, or brittle due to the severe cold in winter, leading to a loss of confidence in rubber products and the closure of many factories. The solution to this problem was evolved by Charles Goodyear, who in 1839 accidentally discovered vulcanization, a process of heating rubber with sulphur at high temperature to improve its strength and elastic properties. Hancock in the UK also made a similar discovery and patented the same in 1843. As vulcanization could eliminate most of the limitations of rubber products, its discovery is considered to be one of the landmarks in the evolution of the rubber industry. Another spectacular development in the history of rubber was the invention of the pneumatic tyre, first patented in 1845 by William Thompson and later in 1888 by John Dunlop. With these developments the industrial demand for rubber increased and the consumption in the UK alone rose from 307 tonnes in 1840 to 13200 tonnes in 1890 and 25664 tonnes in 1900. The consumption in the USA was 22026 tonnes in 1900.

14.4 Plantation Rubber

During the 19th century most of the rubber for the world rubber industry came from *Hevea brasiliensis*, *Ficus elastica* and *Castilla elastica* which grew wild in the forests of Central and South America, India, and Africa. South and Central America was the main source, contributing about 71% in 1876. The growing rubber industry in Europe and America found it difficult to sustain itself with the limited supply of wild rubber from tropical America, and hence had to widen the sources of supply of its most important raw material. The idea of raising plantations of rubber in the tropical East thus evolved, and the colonial powers initiated a scheme for introducing rubber as a plantation crop in South East Asia, having a more or less similar agroclimate as in the Amazon valley. The domestication of rubber in the East was entirely a project initiated and funded by the then government of India through the East India Company, and Sir Clements Markham of the India Office in London, who had successfully transferred cinchona plants from Peru to India during the early 1860s, took the real initiative for the programme. Based on a contract with the Royal Botanic Gardens, Kew, Sir Robert Wickham collected 70,000 seeds of rubber from the Amazon valley and transported the same to Kew Gardens in June 1876. Only 2,700 seemed to have germinated, and from this a shipment of 1,919 seedlings packed in portable greenhouses was sent to Ceylon, of which 90% survived. A shipment of 18 seedlings was sent to the Botanic Gardens at Bogor, Indonesia, of which only two survived, and only one of the 50 seedlings

sent to Singapore survived. The seedlings that arrived in Ceylon were planted in October 1876 at Heneratgoda. Another collection of rubber seeds from the Amazon valley was made by Robert Cross, also in 1876, who brought over 1000 seeds to Kew in November.

During the early days of rubber, Ceylon became the centre of activity with the Heneratgoda Botanic Gardens in Colombo being the major supplier of rubber seeds and seedlings for domestic distribution and for export. Experimental plantings were done in the British colonies of Malaya, Ceylon, and India, and also in the Dutch East Indies. The enthusiasm among planters was initially very low as the commercial potential of the new crop was yet to be fully established. By 1900 the total planted area was only 2800 ha (Malaya – 2400 and Ceylon – 400). But with the outbreak of coffee rust in Malaya and Ceylon, and the subsequent slump in coffee prices during the 1890s, coffee growers started replanting coffee plantations with rubber. The development of the automotive and cable industries during the early twentieth century and the consequent high demand for rubber provided the required stimulus for the expansion of the rubber plantation industry. By 1910 plantation rubber accounted for about 50% of the total global consumption of rubber. By 1940 rubber plantations almost phased out wild rubber from Amazonia. Currently over 10 million ha of rubber plantations exist in over 20 countries, mostly in tropical Asia and Africa, producing around 10 million tonnes of natural rubber, meeting approximately 44% of the global demand for rubber, the rest being met by synthetic rubbers, developed during and after the Second World War.

14.5 Rubber Cultivation

14.5.1 The Para Rubber Tree

Natural rubber is commercially produced from the Para rubber tree, *Hevea brasiliensis*. It is a sturdy tree which attains a height of about 30 m and has a straight trunk with light grey bark. Branches are usually developed to form an open leafy crown. Rubber is a constituent of the latex, a milky substance produced in the laticiferous tissues. Although latex is present in almost all parts of the tree, the laticifers exploited commercially are those in the bark, which are also called latex vessels. The rubber tree is deciduous and winters from December to February in most parts of South East Asia. The bark of the tree has special significance for the commercial exploitation of the tree. All the tissue outside the cambium is collectively termed bark. The mature bark has an inner zone which is soft, an intermediate zone which is hard, and an outermost protective region consisting of layers of cork cells. When the tree is tapped for extraction of latex, a thin layer of bark consisting of the hard bast and a major part of the soft bast is removed. A thin layer of the innermost region of the soft bast along with the vascular cambium, known as the residual bark, is left intact, wherefrom bark renewal takes place which can be exploited later. The original genetic material of the rubber tree, introduced to South East Asia, was

reported to have an average yield of 200–300 kg per ha per year [7]. Now there are rubber clones with annual production potential of 3500 kg per ha [8]. This improvement has been achieved through successive breeding and selection.

14.5.2 Agro-climatic Requirements

The original habitat of the rubber tree in the Amazon valley is situated within 5° latitude and at altitudes less than 200 m. Naturally, the trees evolved in this environment prefer warm and humid weather [9]. The climatic conditions suitable for optimum growth of rubber trees include an annual rainfall of not less than 2000 mm, evenly distributed without any marked dry spell, a maximum temperature of 29–34°C and a minimum of 20°C or more, high atmospheric humidity of around 80% with moderate wind, bright sunshine amounting to about 2000 hours per year at the rate of six hours per day throughout the year [10]. Rubber needs a well-drained, fairly deep, loamy soil with a pH value of 4.5–6.0. South East Asia is particularly suitable for rubber cultivation as are countries in West Africa. Although not ideally suited, rubber is now successfully cultivated even up to latitudes of 25° in countries such as India and China.

14.5.3 Planting

Rubber is planted at a typical density of 450–500 trees per ha. Although the plants were earlier raised directly from seeds, the present propagation practice is vegetative and known as bud-grafting, in which buds taken from a selected high yielding plant are grafted to seedling stocks (Figure 14.1 and Figure 14.2), and the buds allowed to grow into trees which will have all the characteristics of the mother tree. All the trees derived from a single mother tree through vegetative propagation constitute a clone. Recently tissue culture methods have been evolved for rubber [11], but not yet commercialized. The young budded plants are allowed to grow, preferably in plastic bags, in a nursery in the first year and then transplanted to the field. Soil fertility is maintained by the use of fertilizers and by growing leguminous cover crops. Compared to many other crops, the fertilizer requirement of rubber is very limited, since nutrient removal through the crop is very low and as the cultivation practices allow for nutrient recycling through litter disintegration and nitrogen fixation by the legume cover. The trees (Figure 14.3) become ready for harvesting in 5–7 years when they attain a girth of 50 cm at a height of 1.25 m from the ground.

14.5.4 Disease Control

Rubber trees are affected by a number of diseases involving roots, stems, and leaves. However, effective control measures have been evolved for all these diseases, except the South American Leaf Blight, caused by the fungus *Microcyclus ulei*, which has caused extensive damage to rubber plantations in South America. Fortunately Asia and Africa are free from this disease

Figure 14.1 A rubber seedling nursery after bud grafting.

Figure 14.2 Bud-grafting.

and quarantine regulations are in force to prevent any accidental introduction of the disease to this region. The other major leaf diseases are abnormal leaf fall caused by different species of *Phytophthora*, powdery mildew caused by *Oidium heveae*, *Corynespora* leaf disease, and *Gloesporium* leaf disease. Pink

Figure 14.3 Mature rubber plantation.

disease caused by *Corticium salmonicolor* is a major problem affecting the stem, while brown and white root diseases are the common problems affecting the root system. Copper based fungicides are very effective in controlling most of the diseases [12].

14.5.5 Tapping and Collection of Crop

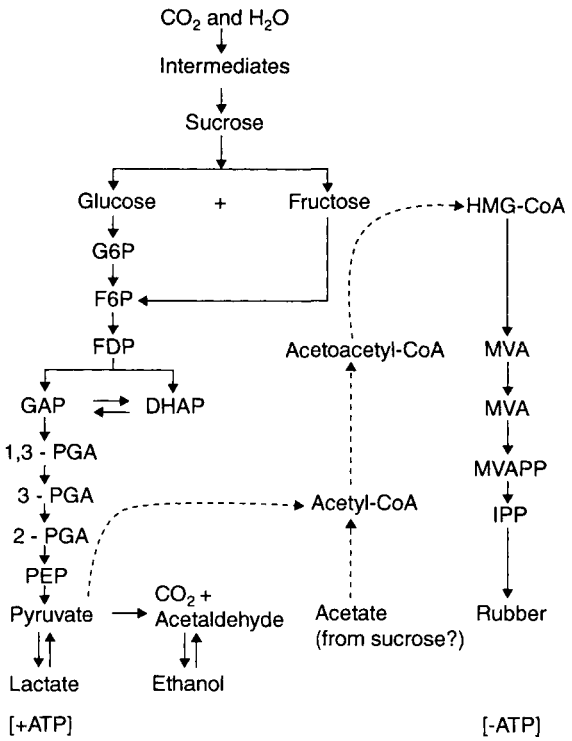
To extract latex from the tree, the latex vessels in the bark are opened by a process called tapping, in which a thin shaving of bark, about 1 mm thick, is removed with a sharp knife, to a depth very close to the cambium, but without injuring it (Figure 14.4). The tapping cut is made at an angle of approximately 30° to the horizontal from high left to low right. As latex vessels run in a spiral up the trunk at an angle of 2–7°, this cuts the maximum number of vessels for a given length of cut. Latex is present in the vessels at a high hydrostatic pressure called turgor of approximately 1–1.9 MPa [13]. As turgor is maximum just before sunrise, tapping is carried out early in the morning to realize maximum yield of latex. Tapping is done at regular intervals, usually once in 2–3 days, working down the trunk. There are variations regarding length of tapping cut and frequency of tapping. Common systems are half spiral alternate daily, i.e., a cut extending half way round the circumference, made every other day, abbreviated as 1/2S d/2. As high intensity tapping is believed to cause problems such as tapping panel dryness in high yielding clones, lower frequency tapping systems

Figure 14.4 Tapped rubber tree.

such as 1/2S d/3 and 1/2S d/4 are being recommended. Yield varies with the clone, age of the tree, fertility and moisture content of the soil, climatic conditions, tapping system, skill of the tapper, etc. A tree can be economically tapped for a period of 20–25 years, after which it is 'slaughter tapped', i.e., intensively tapped for about 2–3 years before being replanted. After tapping, latex flows down the cut which is channelled into a plastic/ceramic cup through a short metal spout. The daily task of a tapper is usually 300–400 trees, which is completed in about 3 hours. Latex flow will continue for about 3 hours after which it stops due to a process of plugging of latex vessels. Then the cups are emptied into buckets and the latex carried to collection centres or processing factories. Very often the tree will still be yielding at the time of latex collection. In such cases, the collection cup, after emptying, is replaced on the tree to receive the late drippings. At the time of the next tapping, this latex will have spontaneously coagulated and the 'cup lump' thus formed is collected by the tapper. Before the next tapping, the coagulated latex residue from the previous tapping is peeled off from the tapping cut as 'tree lace'. If there is any spillage of latex, it is left to coagulate and is collected occasionally from the tree trunk as 'tree scrap' and from the ground as 'earth scrap'. The cup lump, tree lace, tree scrap, and the earth scrap together make the 'field coagulum' which constitutes about 20% of the crop. The remaining 80% is latex. There are certain chemical preparations that can stimulate latex flow causing increased yield. The most widely used stimulant is 2-chloroethylphosphonic acid which was first introduced by Abraham [14]. This stimulant acts by slowly releasing ethylene gas through hydrolysis, which is thought to inhibit the latex vessel plugging process.

14.6 Biosynthesis of Rubber

Natural rubber is a polymer formed by linking thousands of isoprene units together. The various steps in the synthesis of rubber from sucrose are well established and the pathway is illustrated in Figure 14.5. Three major stages have been identified: (1) generation of acetyl co-enzyme A, (2) conversion of acetyl co-enzyme A to isopentenyl pyrophosphate (IPP) via mevalonic acid and, (3) polymerization of IPP to rubber. Sucrose is the primary source of acetate and acetyl co-enzyme A, essential for the biosynthesis of rubber. Acetate forms the basic precursor of rubber synthesis in all rubber yielding plants. The rubber from Hevea differs from the majority of isoprenoid compounds in two respects. It has high molecular weight, which varies from one hundred thousand to a few million, and the geometric configuration around the double bond is exclusively cis-1, 4.



G6P	- Glucose-6-phosphate	PEP	- Phosphoenol pyruvate
F6P	- Fructose-6-phosphate	ATP	- Adenosine triphosphate
FDP	- Fructose-1,6-diphosphate	Acetyl-CoA	- Acetyl-coenzyme A
GAP	- Glyceraldehyde-3-phosphate	Acetoacetyl-CoA	- Acetoacetyl-coenzyme A
DHAP	- Dihydroxyacetone phosphate	HMG-CoA	- β-Hydroxy-β-methylglutaryl-coenzyme A
1,3 PGA	- 1,3-Phosphoglyceric acid		
3-PGA	- 3-Phosphoglyceric acid	MVA	- Mevalonic acid
2-PGA	- 2-Phosphoglyceric acid	VMAP	- Mevalonic acid 5-phosphate
IPP	- Isopentenyl pyrophosphate	MVAPP	- Mevalonic acid 5-pyrophosphate

Figure 14.5 Pathway of rubber biosynthesis (From ref.15).

14.7 Chemistry of Latex

Natural rubber latex is a colloid, the dispersed phase being predominantly rubber and the dispersion medium water. Fresh latex has a specific gravity of 0.96–0.98 and a pH in the range of 6.5–7.0. In addition to rubber and water, latex contains small quantities of proteins, resins including fats, fatty acids, other lipids, sterol and sterol esters, carbohydrates, and mineral matter. Fresh latex is composed of rubber (30–40%), proteins (1–1.5%), resins (1.5–3%), mineral matter (0.7–0.9%), carbohydrates (0.8–1%), and water (55–60%).

The size of the rubber particles range from 0.02–3.0 μm and are mostly spherical in shape, and the particles are strongly protected by a film of adsorbed proteins and phospholipids [16]. The other particles in latex comprise lutoids and Frey-Wyssling particles. The lutoids are subcellular membrane bound bodies ranging in size from 2–5 μm, containing a fluid serum known as B-serum, which is a destabilizer for rubber particles. Frey-Wyssling particles are spherical, larger in size and are yellow coloured. Methyl-l-inositol (quebrachitol), sucrose, and glucose are the major carbohydrates in latex. About 20% of the available proteins in latex are adsorbed on the rubber particles, an equal quantity found in the B-serum, and the remainder in the latex serum. The adsorbed proteins and phospholipids impart a negative charge on the rubber particles, thereby improving the stability of the colloid. The lipids associated with rubber and non-rubber particles play a key role in the stability and colloidal behaviour of latex. Latex also contains amino acids, nucleotides, and low molecular weight thiols. The nucleotides are cofactors and intermediates in the biosynthesis of rubber.

14.8 Primary Processing

The fresh latex and the various forms of field coagulum are not suitable for storage and marketing as these are likely to deteriorate on storage. The presence of carbohydrates and other non-rubber constituents makes latex a suitable medium for the growth of microorganisms such as bacteria and yeast. While bacteria cause conversion of carbohydrates into volatile fatty acids and proteins into simple compounds, the yeast causes fermentation of the carbohydrates. These reactions cause spontaneous coagulation and putrefaction of latex. Thus, fresh latex on keeping slowly undergoes thickening and ultimately coagulates into a lump. Putrefaction of proteins causes the formation of foul smelling gases such as hydrogen sulphide and sulphur dioxide. Fermentation leads to the production of large quantities of carbon dioxide which causes the coagulum to expand. Also prolonged storage of the wet coagula leads to deterioration of the rubber. Hence, both fresh latex as well as the field coagula have to be processed into forms suitable for long-term storage, marketing, and further processing into a finished product. The process of conversion of fresh latex and/or field coagula into a suitable marketable form of raw rubber is called primary processing, as another stage of processing is necessary to convert the

raw rubber into a finished product. The various marketable forms of raw rubber include preserved field latex and concentrated latex, ribbed smoked sheet (RSS) and air dried sheet (ADS), block or technically specified rubber (TSR), pale latex crepe (PLC) and estate brown crepe (EBC), and other low grade crepe made from field coagula.

14.8.1 Preserved and Concentrated Latex

Rubber products such as gloves, condoms, rubber bands, balloons, foam mattresses, elastic thread, etc. are made directly from latex, for which about 10% of the global production of NR is processed as preserved and concentrated latex. Preservation of latex aims at preventing bacterial activity and enhancing its stability. It is achieved by using preservatives such as ammonia. Apart from being a good bactericide, ammonia enhances the colloidal stability of latex and deactivates certain harmful metal ions. To be effective as a preservative, ammonia is used at 0.7–1.0% by weight of latex. During storage, the higher fatty acid esters present in latex get hydrolysed into ammonium soaps, which improve the mechanical stability of latex. However, the pungent smell of ammonia, the low stability of latex in the presence of zinc oxide, and the longer gelling time of latex foam are disadvantages. Hence, many low ammonia preservative systems have been developed, in which the concentration of ammonia is reduced to around 0.2% and supplemented with secondary preservatives such as tetramethyl thiuram disulphide(TMTD)-zinc oxide combination, zinc diethyl dithiocarbamate, boric acid, etc. Of these, a combination of 0.2% ammonia, 0.0125% each of TMTD and zinc oxide and 0.05% of lauric acid (LATZ system) is the most promising.

Concentration of latex is necessary because of the preference by the latex products manufacturing industry for latex with high dry rubber content (DRC), and for transport economy and purification of the latex. The processes for concentration include evaporation, electrodecantation, creaming, and centrifuging. Evaporation removes only water and hence the ratio of non-rubber constituents to rubber, and the particle size distribution remain unchanged. However, the other three processes involve the partial removal of non-rubber materials and smaller rubber particles. Centrifuging and creaming are more popularly used for the production of concentrated latex.

The principle of creaming and centrifuging involves the application of Stokes' Law [17]. In any dispersion, the dispersed particles cream or sediment under the influence of gravity and the velocity of creaming depends on several factors and is stated mathematically as:

$$V = 2g(Ds - Dr)\, r^2 / 9\acute{\eta} \tag{14.1}$$

Where V is the velocity of creaming (m/s)
g is the acceleration due to gravity (m/s^2)
Ds, Dr are the densities of serum and rubber particles, respectively (g/cm^3)
r is the effective radius of the particle (cm) and
$\acute{\eta}$ is the coefficient of viscosity of serum (centipoise)

In the creaming process, the creaming agent such as sodium alginate forms an adsorbed layer over the rubber particles, which reduces the Brownian movement and the intensity of the negative electric charge on the particle surface, thereby promoting temporary agglomeration of particles. Thus, the effective size of the particle increases, favouring faster creaming. In the centrifugal process, when the latex enters the machine, rotating at a high speed of around 7000 rpm, the centrifugal force, which as several thousand times larger than the gravitational force, replaces g and causes rapid separation of the latex into cream and skim. In creaming, the skim is almost clear serum and is therefore discarded. However, in the centrifugal process, the skim contains 2–8% rubber, which is recovered by coagulation using dilute sulphuric acid and processed as skim rubber, which is a low quality rubber. Concentrated natural rubber latex is marketed to strict technical specifications. The current ISO specifications for latex concentrates are given in Table 14.2.

14.8.2 Ribbed Smoked Sheet

This is the preferred form of processed natural rubber in smallholdings in countries such as India and Thailand. The process is simple, relatively less expensive, and is highly viable even when the quantity of available latex is small. Fresh field latex, after sieving to remove foreign matter and bulking to make it uniform in quality, is diluted to 12.5–15% DRC. Dilution improves the colour and transparency of the sheet, makes the sheeting operation easier, and allows denser impurities to sediment on standing for 10–15 min. Sodium bisulphite is added to latex at the rate of 1.2 g per kg DRC to prevent surface darkening on the wet sheet caused by enzyme catalyzed oxidation of phenolic components in latex. The diluted latex is then transferred to coagulation tanks or pans and coagulated with dilute formic or acetic acid. These acids are preferred because of their volatile and non-corrosive nature. After a few hours or the next day, the thick slab of coagulum is squeezed between a pair of steel or cast iron rollers to remove water and to make into a sheet of approximately 3 mm thickness. The final set of rollers is grooved to introduce ribbed markings on the sheets. These markings increase the surface area of sheet and hence facilitate drying. The wet sheets are soaked in a 0.1% solution of paranitrophenol (PNP) for a few minutes to prevent mould growth on dry sheets during storage. The sheets are then allowed to drip for a few hours before loading into the smoke house for drying.

Sheet rubber is ideally dried in smoke houses or hot air chambers, and the dried sheet thus obtained are known as ribbed smoked sheet (RSS) or as air dried sheet (ADS) respectively. Partial drying in sun followed by smoke drying is widely practised in smallholdings. Several types of smoke houses such as those with a furnace inside or outside the chamber, batch type, or continuous type are in operation [18]. The temperature in the smoke house is maintained in the range of 40–60°C. The sheets are held on wooden or bamboo rods (beroties) placed on trolleys which move on a central rail track in a large tunnel type smoke house. In the smaller type of smoke houses, (Figure 14.6 and Figure 14.7) the beroties are placed on a framework inside the chamber. Drying

Table 14.2 ISO 2004 : 1997 (E) (Specifications for concentrated natural rubber latex).

Characteristic	Type HA	Type LA	Type XA	Type HA creamed	Type LA creamed	Method of test
Total solids content[1], % (m/m), min.	61.5	61.5	61.5	66.0	66.0	ISO 124
Dry rubber content[1], % (m/m), min.	60.0	60.0	60.0	64.0	64.0	ISO 126
Non-rubber solids[2], % (m/m), max.	2.0	2.0	2.0	2.0	2.0	–
Alkalinity (as NH_3), % (m/m), calculated with respect to the latex concentrate	0.60 min	0.29 max	0.30 min	0.55 min	0.35 max	ISO 125
Mechanical stability, seconds, min.	650	650	650	650	650	ISO 35
Coagulum content, % (m/m), max.	0.05	0.05	0.05	0.05	0.05	ISO 706
Copper content, mg/kg of total solids, max.	8	8	8	8	8	ISO 8053
Manganese content, mg/kg of total solids, max.	8	8	8	8	8	ISO 7780
Sludge content, % (m/m), max.	0.10	0.10	0.10	0.10	0.10	ISO 2005
Volatile fatty acid (VFA) number, max.	0.20	0.20	0.20	0.20	0.20	ISO 506
KOH number, max.	1.0	1.0	1.0	1.0	1.0	ISO 127

[1] The requirement is for either total solids content or dry rubber content.

[2] The difference between the total solids content and dry rubber content.

Figure 14.6 Trolley with sheet rubber.

Figure 14.7 Smoke house.

of sheets takes four to six days in smoke houses. The dried sheets are visually examined and graded adopting the norms prescribed by the International Rubber Quality and Packing Conference under the Secretariat of the Rubber Manufacturers' Association (RMA) and described in the 'Green Book'. Grading is done on the basis of colour, transparency, presence of mould, oxidized spots, blisters, bubbles, dirt, sand and other foreign matter, degree of drying, tackiness, etc. There are six grades of sheet rubber which are designated as RMA or RSS 1X and RSS 1 to RSS 5. RSS 1X is the best and RSS 5 the lowest quality. Sheets of the same grade are packed together into a bale.

14.8.3 Pale Latex Crepe and Sole Crepe

These two are superior quality forms of natural rubber, prepared from fresh latex under controlled conditions. Both are light coloured and hence are produced from latex after either removing or bleaching the naturally occurring yellow carotenoid pigments. These forms of NR are preferred in products where light colour is important. Latex from certain selected clones such as PB 86 and RRIM 600, which give relatively white latex with minimum β-carotenes and are less susceptible to enzymic discolouration, is preferred for production of PLC. Precoagulation of latex is avoided preferably by the addition of sodium sulphite. The latex, after bulking is diluted to 20% DRC and the yellow pigments are bleached with 0.05% of tolyl mercpatan or its water souluble alkali metal salt [19]. Alternatively, the pigments are removed by fractional coagulation where about 1 g of acetic acid or oxalic acid is added to every kg DRC of latex followed by stirring and allowing it to remain undisturbed for 1–2 hours. About 10% of the rubber coagulates along with the bulk of the yellow pigments. The coagulum is removed and the remaining fraction of latex is fully coagulated and processed into crepe with thorough washing. The thin crepe, 1–2 mm thick, is dried in air at a maximum temperature of 35°C for about two weeks. Very often, a combination of fractional coagulation and bleaching is used. Pale crepe is graded visually according to the Green Book as in the case of RSS. The main criterion is colour and the grades are PLC 1X, PLC 1, PLC 2, and PLC 3. Sole crepes are used in the footwear industry and are made by laminating plies of pale crepe to the required thickness and consolidating them by hand rolling and finally through even speed rollers.

14.8.4 Field Coagulum Crepe

Cup lump, tree lace, and other coagulated forms of the crop can be processed into crepe after soaking in water to make them soft and to remove surface contaminants. The soaked material is then passed through a battery of crepe rollers. Proper blending is carried out on the rollers to ensure uniformity. Simultaneously, thorough washing is done by providing running water in every crepe roller. The crepe is dried in drying sheds at ambient or slightly elevated temperature. Grading is done visually as per Green Book and the main criterion is again colour. Different grades are available in each type.

14.8.5 Technically Specified Rubber

With the availability of synthetic rubbers in compact bales and as per technical specifications, the disadvantages of sheet and crepe rubbers, available in irregularly shaped bales and with visual grading system, became very evident. Consequently new methods of processing and presentation were developed to market NR as technically specified rubber in compact bales, wrapped in polyethylene film. TSR is now produced in almost all NR producing countries and are marketed under different names such as Standard Malaysian Rubber (SMR), Standard Indonesian Rubber (SIR), Standard Thai Rubber (STR), Indian Standard Natural Rubber (ISNR), etc. The advantages of TSR include assurance of quality for important technical parameters, consistency in quality, minimum space for storage and clean and easy to handle packing. This method enables NR producers to process both latex and field coagulum using almost the same set of machinery and to minimise the processing time to less than 24 hours. Although different methods were developed to produce TSR, all these processes involve certain common steps such as coagulation of latex, soaking of field coagulum in water, size reduction, drying, baling, testing, grading, and packing. Differences among commercial processes exist in the method of coagulation or in the machinery used for crumbling the coagulum. Typical flow diagrams for processing of latex and field coagulum into TSR are given in Figure 14.8.

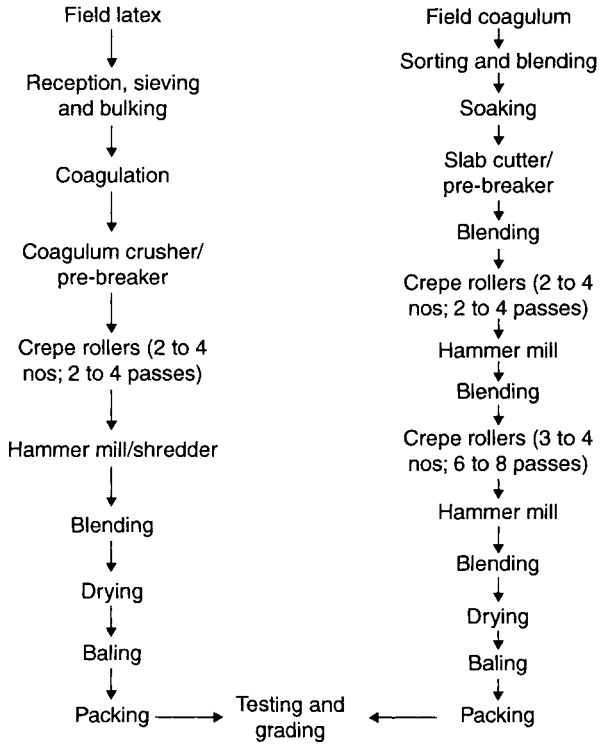


Figure 14.8 Flow diagram for processing latex and field coagulum into technically specified rubber.

The major challenge in TSR processing is ensuring consistency in quality. Introduction of slab cutters or pre-breakers in the initial size reduction stage, use of macro- and micro-blending tanks, transfer of raw material from one tank to another by bucket elevator system, and loading of final crumbs to the drier boxes with a slurry pump through a vibrating perforated screen and hopper are some of the recent developments in TSR production aimed at improving consistency in quality and reducing cost. The specifications for TSR have been revised several times depending upon the requirements of the consuming industry. The current ISO specifications for TSR are given in Table 14.3.

Table 14.3 ISO 2000 : 1989 (E) (Specifications for raw natural rubber).

Characteristic	Limits for rubber grades						Test method
	CV	L	5	10	20	50	
	Colour code						
	Green	Green	Green	Brown	Red	Yellow	
Dirt content, %(m/m) retained on 45 μm sieve, max.	0.05	0.05	0.05	0.10	0.20	0.50	ISO 249
Initial plasticity, min.	–	30	30	30	30	30	ISO 2007
Plasticity retention index (PRI), min.	60	60	60	50	40	30	ISO 2930
Nitrogen content, % (m/m), max.	0.60	0.60	0.60	0.60	0.60	0.60	ISO 1656
Volatile matter content, % (m/m), max.	0.80	0.80	0.80	0.80	0.80	0.80	ISO 248 (Oven method at 100 ± 5°C)
Ash, % (m/m), max.	0.60	0.60	0.60	0.75	1.00	1.50	ISO 247
Colour index, max.	–	6	–	–	–	–	ISO 4660
Mooney viscosity, ML(1 + 4) 100°C	60 ± 5*						ISO 289

• As produced. Other viscosity levels may be obtainable on request

14.9 Current Global Status of Production and Consumption

Global production and consumption of natural rubber have been growing, although not steadily, during the past decade, as is seen from Table 14.4 [20, 21]. The variation in annual growth rate in production is mostly contributed by price and agro-climatic conditions. The growth in consumption also has had its influence on production as is evident from the above table.

Production and consumption of synthetic rubbers also have been growing, but at relatively slower rates. In 2009, the total global production and consumption of synthetic rubbers were 11.88 and 12.17 million tonnes respectively. The consumption pattern between natural and synthetic rubbers also has been slowly moving towards the former. The percentage of share of natural rubber in the total global rubber consumption, which remained at around 30% in the 1970s, has increased slowly and at present remains at around 44%. The increased preference for natural rubber has been because of technological factors such as radialization of tyres, relative prices and availability of the two varieties, and of late, the green credentials of the natural polymer.

14.10 Properties of NR

Natural rubber molecule is a linear, unsaturated, long chain aliphatic hydrocarbon polymer identified chemically as cis-1, 4 polyisoprene. As in the case

Table 14.4 Global production and consumption of natural rubber during 2000-10.

Year	Production ('000 tonnes)	Growth, %	Consumption ('000 tonnes)	Growth, %
2000	6764	–	7313	–
2001	7242	7.07	7219	–1.30
2002	7326	1.16	7556	4.67
2003	8020	9.47	7939	5.07
2004	8746	9.05	8718	9.81
2005	8904	1.81	9200	5.53
2006	9791	9.96	9714	5.59
2007	9801	0.10	10224	5.25
2008	10031	2.35	10154	–0.68
2009	9602	–4.28	9547	–5.98
2010	1029	7.17	1067	11.76

of other high polymers, natural rubber too is formed of molecules of different sizes with the weight average molecular weight ranging from 100,000 to about 10 million. A random blend of natural rubber shows a weight average molecular weight in the range of 1–1.5 x 10^6, and a number average molecular weight of 3–5 x 10^5. High molecular weight results in high viscosities. The Mooney viscosity of fresh dry natural rubber is in the range of 55–90. During storage the viscosity of natural rubber increases to 70–100 Mooney units depending on the length of the storage period. The natural storage hardening is believed to be due to the cross-linking reaction between aldehyde groups present in the rubber molecule with the amino groups of free amino acids and proteins [22–24]. The aldehyde groups can be deactivated and storage hardening reaction can be inhibited by treating the rubber with 0.15% hydroxylamine salt. This is the technique used for the production of viscosity stabilized rubber grades, also known as constant viscosity (CV) rubbers.

Because of the high structural regularity, natural rubber crystallizes spontaneously when stored at low temperatures, or when it is stretched. Unstrained sample shows a maximum rate of crystallization at about -26°C. The maximum degree of crystallinity attained is about 25–30 %. The unstretched raw rubber shows a crystalline melting point (T_m) of +25°C [25–26]. The strain induced crystallization behaviour gives natural rubber high tensile strength even in pure gum or vulcanizates filled with non-reinforcing fillers. Glass transition temperature of natural rubber is higher than that of cis-1, 4 polybutadiene.

The density of natural rubber is about 0.913 g per cc, and its bulk density is about 0.85 g per cc. The rubber hydrocarbon content of raw natural rubber is about 94%. The presence of small quantities of non-rubber constituents such as proteins, fats, fatty acids, carbohydrates, and mineral matter in natural rubber influences its physical and chemical properties.

A small percentage of rubber undergoes cross-linking even when present within the latex vessels of the tree. This is evident from the 5–10% gel content (microgel) in freshly prepared natural rubber. The gel content increases during storage and it is known as the macrogel. The macrogel is responsible for increased viscosity of natural rubber during storage. The macrogel breaks down almost completely during mastication of the rubber, and dissolves completely in aliphatic and aromatic solvents, chlorinated hydrocarbons, tetrahydrofuran and carbon disulphide.

Coagulated and dried natural rubber is highly elastic at ambient temperature. On subjecting the dry rubber to intensive mechanical shearing it becomes progressively more plastic. This process is known as mastication. Masticated rubber is capable of flow, and therefore it can be shaped. The efficiency of mastication is temperature dependent and its minimum is at about 100°C [27]. Staudinger and Boundy [28] suggested that mastication involves the rupture of the rubber molecules due to the shearing forces. Efficiency of mastication can be improved by the addition of small quantities of chemicals known as peptizing agents (e.g. zinc pentachlorothiophenate).

The high molecular weight and the polymer chain entanglements make the dissolution of natural rubber in solvents difficult. Masticated natural rubber

with a solubility parameter of about 16.5 $MPa^{1/2}$ dissolves in a number of solvents of similar solubility parameter.

The chemical reactivity of natural rubber is influenced by the presence of double bond in its molecular backbone. The presence of methyl group in the cis-1, 4-polyisoprene further enhances the reactivity of the double bond. Thus natural rubber is more reactive than polybutadiene, and poly(styrene butadiene) rubber to a number of chemicals, including the vulcanization chemicals.

14.11 Blends of Natural Rubber

14.11.1 Blends of Natural Rubber with Thermoplastics

Raw natural rubber shows high green strength by virtue of strain-induced crystallization. Though natural rubber in the raw form is used in a few applications such as adhesives, binders, and sole crepe, it is not truly thermoplastic, primarily due to its high molecular weight [29].

Mixing of natural rubber with polyolefin is one of the methods used to prepare thermoplastic natural rubber (TPNR). TPNR behaves like vulcanized rubbers at ambient conditions, but at elevated temperatures they melt and flow like a thermoplastic material. Thus TPNR could be processed using conventional thermoplastic processing machinery without requiring vulcanization, and these materials could be reprocessed. Thus there is low level of wastage, as scrap too can be recycled.

14.11.2 Preparation of Thermoplastic Natural Rubber

Thermoplastic natural rubber blends are prepared by blending natural rubber and polyolefins, particularly polypropylene (PP), in an internal mixer. Immediately after dumping from the internal mixer, the blend is sheeted on a two roll mill to a thickness of 4–8 mm. The mixing parameters include a temperature in the range of 175–185°C and a total mixing time of about six minutes. Very high temperature of mixing is undesirable, as natural rubber may degrade at temperatures above 200°C. The thermoplastic elastomeric nature of the blends is because of their two phase morphology. The hard phase that may be glassy or microcrystalline constitutes the pseudo-crosslinks or tie points, and these disappear upon heating. The elastic properties of the thermoplastic natural rubber blends are considerably improved if the rubber is partially cross-linked during blending. This process is known as dynamic cross-linking [30, 31]. The popular cross-linking agents used in NR/PP blends include dicumyl peroxide and sulphur donor systems.

14.11.3 Properties and Applications of TPNR

TPNR covers a wide range of material properties and has a correspondingly wide range of potential applications. TPNR in the 55–95 Shore A hardness

range can be used to replace vulcanized rubbers and flexible plastics for applications in footwear, sporting goods, seals, mountings, and a wide range of moulded and extruded goods. Due to the presence of PP as a continuous phase, ozone resistance of TPNR is better than that of NR [32]. Grades of TPNR have been used for automotive bumpers, car radiator grills, floor tiles, castor wheels, handles, and grips.

14.12 Modified Forms of Natural Rubber

14.12.1 Introduction

As natural rubber is a product of nature, its properties are determined by the biochemical pathway by which the polymer is synthesized in the plant. In the case of natural rubber the polymerization process cannot be tailored like that of synthetic rubbers. The only option to modify natural rubber is after it has been harvested from the tree. The important modified forms of natural rubber include hydrogenated natural rubber, chlorinated natural rubber, hydro-halogenated natural rubber, cyclized natural rubber, depolymerised liquid natural rubber, resin modified natural rubber, poly(methyl methacrylate) grafted natural rubber, poly(styrene) grafted natural rubber, and epoxidized natural rubber [33, 34]. Thermoplastic natural rubber prepared by blending natural rubber and PP is considered as a physically modified form of natural rubber.

A few of the modified forms of natural rubber which have assumed commercial and/or scientific importance are detailed below.

14.12.2 Hydrogenated Natural Rubber

Hydrogenation of natural rubber is still a scientific curiosity. Fully hydrogenated natural rubber, due to the removal of its unsaturation is unaffected by chemical reagents that normally attack unmodified natural rubber. However, the fully hydrogenated natural rubber cannot be vulcanized in the conventional manner. Platinum black was used as the catalyst for the production of hydrogenated rubber $(C_5H_{10})_x$ from dilute solutions of purified rubber. More recently Burfield *et al.* [35] achieved complete hydrogenation in solution after one hour at 28°C using homogeneous two component catalyst systems based on a variety of nickel and cobalt compounds in combination with triiosobutylaluminum. Potential applications considered for hydrogenated natural rubber are in the cable industry, and for the preparation of adhesive compositions.

14.12.3 Chlorinated Natural Rubber

Chlorination of natural rubber in solution, in latex, or in solid form, results in substitution, addition, and cyclization reactions [34]. Kraus and Reynolds [36] have identified the following three distinct stages in the reaction of gaseous chlorine with natural rubber dissolved in carbon tetra chloride at 80°C:

$$-C_{10}H_{16}- + 2Cl_2 \rightarrow -C_{10}H_{14}Cl_2- + 2\,HCl$$

$$-C_{10}H_{14}Cl_2- + 2Cl_2 \rightarrow -C_{10}H_{13}Cl_5- + HCl$$

$$-C_{10}H_{13}Cl_5- + 2Cl_2 \rightarrow -C_{10}H_{11}Cl_7- + 2\,HCl$$

The product after the first stage contains 35% chlorine, and is rubbery. The second stage product that contains 57% chlorine has no unsaturated groups. Further chlorination results in the final product containing 65.5% chlorine, with the empirical formula $-C_{10}H_{11}Cl_7-$. The final product of chlorination of natural rubber is a pale cream or off-white thermoplastic powder, which is inflammable and highly resistant to chemical attack.

It is also possible to chlorinate natural rubber in the latex stage [37]. The advantages of latex stage chlorination are the lower viscosity of the reaction system, better heat dissipation, and retention of high molecular mass in the product. In one of the methods for the chlorination of natural rubber in the latex stage proposed by van Amerongen [38] the latex is colloidially stabilized against acidification using 2 pphr of non-ionogenic ethoxylates, and 3–5 pphr cationic stabilizers of quaternary ammonium type. The stabilized latex is then strongly acidified with either concentrated hydrochloric acid or gaseous hydrogen chloride. Gaseous chlorine is then passed through the latex at a given temperature until the desired degree of chlorination has been attained.

The main use of chlorinated natural rubber is for chemical and heat-resistant paints and coatings. It is also used in adhesive formulations, printing inks, paper coatings, and textile finishes.

14.12.4 Cyclized Natural Rubber

Cyclized natural rubber was first prepared by Leonhardi in 1791 by treating natural rubber with sulphuric acid. At present there are two main routes for the commercial production of cyclized natural rubber; by using sulphuric acid (or its derivatives such as p-toluene sulphonic acid), or by using chlorostannic acid. Cyclization reaction can be carried out in solution and in latex form [39, 40]. The applications of cyclized natural rubber are in printing inks, surface coatings (for chemical resistance), shoe soling, and for hard mouldings.

14.12.5 Graft Copolymers Based on Natural Rubber

Preparation of graft copolymers by polymerizing vinyl monomers either in natural rubber solution or in latex is a method for chemically modifying natural rubber. The common method to prepare a graft copolymer is to polymerize an olefinic monomer by free radical mechanism within the latex of a polymer derived from another monomer. The result of polymerization is a graft copolymer which comprises the original polymer of the latex as the main chain component, and blocks derived from the added monomer as grafts [41–43]. Graft

copolymers of natural rubber with methyl methacrylate, styrene, and acrylonitrile [44] have been reported. The common methods of free radical polymerization to prepare the graft co-polymers based on natural rubber are: (i) by the use of peroxide based system, and (ii) irradiation with γ-rays.

Poly(methyl methacrylate) graft natural rubber: Poly(methyl methacrylate) grafted natural rubber has been commercially available since the mid-1950s under the trade name Heveaplus. The popular commercial grades are Heveaplus MG 30, Heveaplus MG 40, and Heveaplus MG 49, where the numbers represent the percentage of methyl methacrylate in the grafted copolymer. Heveaplus MG 49 is the most popular grade.

The major use of Heveaplus MG is in adhesives. It gives good bond strength for natural rubber to PVC and therefore, it is particularly useful in shoe manufacturing. It is used as a compatibilizer in plastic-rubber blends. It is also used in applications such as automobile bumpers due to its self-reinforcing nature.

Polystyrene graft natural rubber: Polystyrene can be grafted onto the natural rubber backbone by polymerizing styrene in natural rubber latex. The polystyrene grafted natural rubber is designated as SG rubber. A popular grade of the graft copolymer is SG50, where the number indicates the styrene content in the graft copolymer. The SG 50 graft copolymer is used in place of high styrene (SBR) rubber in rubber compounds for the manufacture of microcellular soling.

14.12.6 Epoxidized Natural Rubber

Epoxidized natural rubber (ENR) is an important modified form of natural rubber whose properties are close to those of special purpose synthetic rubbers. ENR is prepared by reacting natural rubber in latex form with performic acid formed *in situ* by the reaction of formic acid and hydrogen peroxide under controlled conditions. [45, 46]. The double bonds of natural rubber react readily with peracids to yield epoxide groups. Though any level of epoxidation can be achieved, only up to 50 mole percent is used in practice. 50, 25, and 10 mole% epoxidized products are termed ENR-50, ENR-25, and ENR-10. Studies using ^{13}C NMR have shown that the epoxide groups are randomly distributed along the natural rubber backbone [47].

Bulk epoxidised natural rubber can be vulcanised by heating with sulphur and conventional organic vulcanization accelerators, or by heating with peroxides. Baker, Gelling, and Newell [48] have studied the effect of epoxidation on ENR50, ENR 25, and ENR10. As the level of epoxidation is increased, the glass transition temperature (T_g) is raised by approximately 1°C per mole percent epoxidation, and this results in a substantial drop in room temperature resilience for ENR-50, and increase in damping. The Tg increases from –67°C in the case of natural rubber to –47°C for ENR 25, and –23°C for ENR 50. Epoxidized natural rubber retains the cis-1, 4- configuration of natural rubber and hence undergoes strain crystallization and high tensile strength.

As compared to natural rubber, epoxidized grades of natural rubber show improved reinforcement with silica without any coupling agent. Radial tyre tread prepared using formulations based on natural rubber with epoxide

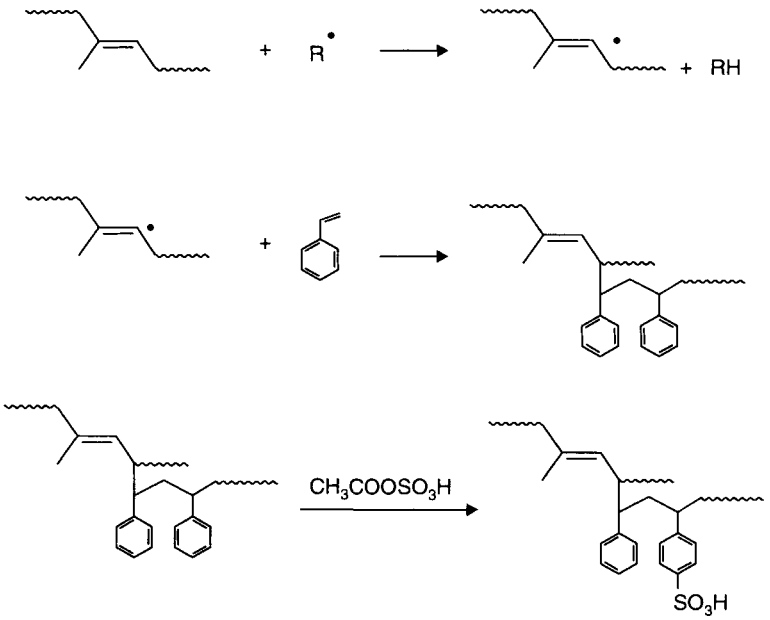
modification shows improved wet traction as compared to the tread based on unmodified natural rubber. Epoxidation of natural rubber reduces its gas permeability, making it suitable for applications such as tyre inner liner. The oil resistance of epoxidized natural rubber is superior to that of polychloroprene rubber [49], and it is almost equivalent to that of nitrile rubber with medium level of acrylonitrile content.

14.12.7 Ionic Thermoplastic Elastomers Based on Natural Rubber

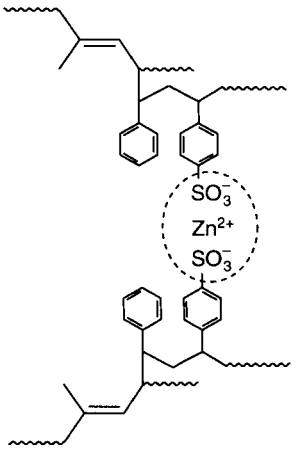
Introduction of small amounts of ionic groups in hydrocarbon polymers exerts a profound effect on their mechanical properties. These ionic groups neutralized with suitable metal ions act as physical cross-links within the polymer matrix. The ionic associations can be thermally relaxed to permit sufficient melt flow at the processing temperature. The ion containing polymer thus behaves as a thermoplastic elastomer having the unique ability to act as cross-linked elastomer at ambient temperature, and to melt and flow at elevated temperatures like thermoplastics [50–53].

The ionomers based on natural rubber were prepared by sulphonating the radiation induced styrene grafted natural rubber (SGNR) in 1, 2-dichloro ethane using acetyl sulphate reagent, followed by neutralization of the precursor acids using methanolic zinc acetate [54–56].

The proposed sulphonation reaction in radiation induced styrene grafted natural rubber, and the structure of the ionomer (zinc sulphonated styrene grafted natural rubber), are shown in Scheme 14.1 and Scheme 14.2. The



Scheme 14.1 Sulphonation reaction on styrene grafted natural rubber.



Scheme 14.2 Ionic interaction between styrene grafted natural rubber units.

ionomeric modification improves the mechanical properties of the styrene grafted natural rubber. The ionomer shows reprocessability too.

14.13 Introduction to the Manufacture of Rubber Products

Rubber has such remarkable and desirable properties that it is being put to use in many engineering applications like bearings, springs, and seals in addition to the manufacture of bulk products like tyres, tubes, belts, hoses, etc. Loads could be safely supported and misalignments accommodated by its ready elastic deformability. The resilience of rubber could be used to advantage in the production of efficient seals.

A simplified flow diagram, which shows the basic steps in the manufacture of products from natural rubber, is shown in Figure 14.9 [57]. Starting with the raw rubber, the first step is usually mastication to soften the rubber by mechanical working and heat [58]. The next step is the mixing or compounding of the softened raw rubber. This is also done in an open two roll mill or in an internal mixer. A wide variety of substances are frequently mixed with natural rubber. To develop a rubber compound the various ingredients to be used are compiled into a recipe. A recipe contains a number of components apart from the base rubber, each having a specific function during processing, vulcanization, or during service of the product. They are: vulcanizing agents, accelerators, activators, fillers, processing aids, antidegradants, and a number of special ingredients.

The processing aids are generally liquids or resinous materials, which mix intimately with rubber and are used as softeners to permit incorporation of fillers, and to modify the flow behaviour of the rubber.

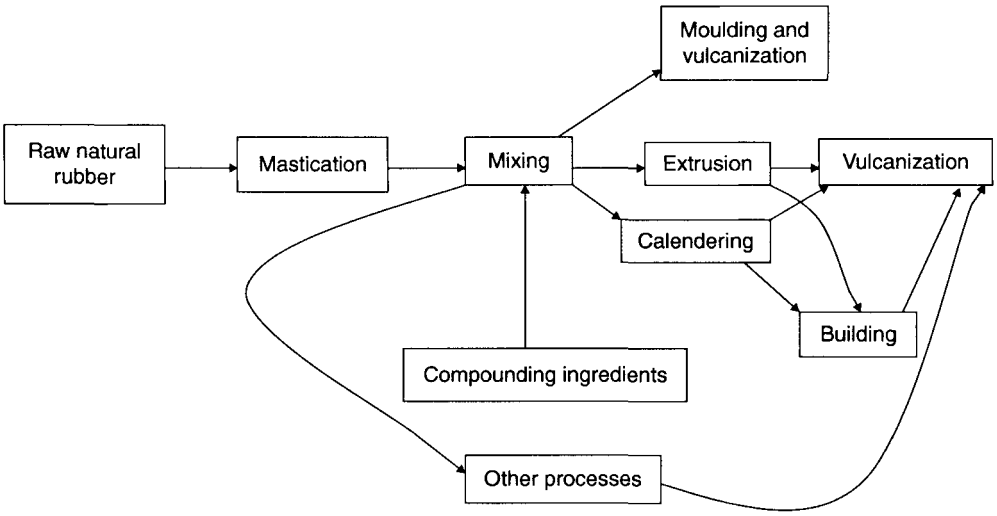


Figure 14.9 Basic steps in the manufacture of rubber products.

Fillers are classified into two groups: reinforcing and non-reinforcing. Reinforcing fillers are used for enhancement of properties like tear strength, abrasion resistance, and tensile strength. Examples of reinforcing fillers are carbon black, china clay, precipitated silica, and silicates. Non-reinforcing fillers are incorporated primarily to cheapen and stiffen the final product. Vulcanizing agents are substances which are added in order that cross-linking may occur subsequently, when the compounded rubber is heated to an appropriate temperature. Sulphur is the principal vulcanizing agent for natural rubber. Accelerators and activators are essential ingredients in the compound to achieve economic rates of vulcanization. A wide variety of organic accelerators are available today. The most widely used vulcanization activators are combinations of a metal oxide (e.g. zinc oxide) and a higher fatty acid (e.g. stearic acid).

Antidegradants are added in rubber compounds in order to increase the ability of the vulcanized products to resist the various deteriorative influences like oxygen, ozone, sunlight, etc., to which it may be subjected to during service.

14.13.1 Processing Methods

Mixing: The general methods for incorporating compounding ingredients into dry natural rubber involve the use of either a two roll mill or an internal mixer (e.g. Banbury mixer). Both in the open mill and in the internal mixer, a definite time, temperature, and order of addition of ingredients should be followed for each batch. [59]

Calendering: The rubber calender is essentially a device for forming rubber sheeting of continuous length and uniform thickness. It is also used extensively for frictioning or skim coating of fabric/cord/wires with rubber for making

plies used in the construction of tires, conveyor belts, etc. Modern calenders have devices for bending the rolls and altering the relative positions of rolls, to ensure more uniform gap between rolls with material between them so as to produce sheets of the most uniform thickness.

Extrusion: Extruders are machines which force rubber through a nozzle or die to a profiled strip of material. Extruders are of two basic types: screw extruders and ram extruders. A screw extruder comprises a feed hopper, a feed screw operating within a barrel, and a die.

The function of the feed hopper is to receive the compound and pass it down the screw flights. The screw is rotated by an electric motor through a reduction gear, and pushes the rubber compound through a barrel into the head where it built up pressure gets relieved by allowing the compound to pass through an orifice or die to form the desired shape. The screw flights have lower volume at the discharge end compared to the feed end to give sufficient compression to eliminate air and to ensure a constant pressure in the head.

In the ram extruder, a ram pushes a quantity of warm compound placed into the cylinder. The operation of this type of machine is intermittent, and the operating costs are higher.

Moulding: Moulding is the operation of shaping and vulcanizing a rubber compound in a mould of appropriate shape by the application of heat and pressure. Depending on the surface quality required in the product, it is necessary to use properly designed and well-constructed hardened metal moulds of suitable surface finish. Depending on the method of introducing the material into the mould, the main moulding processes are compression moulding, transfer moulding, and injection moulding.

Compression moulding: The compression moulding process consists of placing a blank rubber compound inside a two-piece or multi-piece mould, closing the mould and applying pressure between the platens of a press. The excess material flows out of the mould through a suitable provision designed in the mould. Pneumatic tyres are normally cured in a modified compression mould in which a rubber bladder forces the green tyre, and holds it against the mould surface during vulcanization.

Transfer moulding: In transfer moulding the uncured rubber compound is transferred from the loading chamber (pot) of the transfer mould into the actual mould cavity. This technique is suitable for moulding products of a complicated shape, and for anchoring delicate inserts in mouldings.

Injection moulding: Injection moulding is the most popular moulding process for thermoplastic materials because of the speed of the process and the high degree of reproducibility. Recently this technique has also become popular in the case of natural rubber for the mass production of certain rubber products. The cycle time required for the production of mouldings by this technique could be reduced to a few seconds by the design of the formulation of the rubber compound, and the control of the process temperature. The injection moulding technique has the additional advantage of considerably low rejection rates, and lower finishing costs.

14.13.2 Vulcanization Techniques

The finished dry rubber and latex based rubber products can be vulcanized by several techniques depending on the type of rubber compound (dry rubber compound/latex compound), size of the finished product, and its shape and structure. Moulded rubber products are vulcanized by press curing using compression, transfer, or injection moulding presses. The vulcanization techniques other than moulding may be grouped into batch and continuous methods. The batch methods include the use of autoclaves, hot air/gas oven, and hot liquid/water bath. Rubber products may be vulcanized at room temperature by cold curing either by immersion of rubber products in a carbon disulphide solution of sulphur chloride (S_2Cl_2), or by exposure to its vapour.

Products in continuous length such as hoses and cables, are vulcanized either in autoclaves or by continuous vulcanization methods such as high pressure steam filled long jacketed tubes, hot air tunnels, molten salt baths, fluidised bed, or hot liquid baths. Rubber compounds containing polar compounding ingredients may be preheated rapidly by high frequency and microwave techniques before vulcanization by other techniques. High frequency radiation techniques such as gamma irradiation and electron beam irradiation are also used for the vulcanization of rubber products.

14.14 Applications of Natural Rubber

Before World War II (1939–1945) natural rubber was used for practically all rubber applications. Natural rubber is preferred in many products because of its superior building tack, green stock strength, better processability, high strength in non-black formulations, hot tear resistance, retention of strength at elevated temperatures, better resilience, low heat build-up, fatigue resistance, and better dynamic properties. Rubber products are broadly classified as dry rubber products and latex based products [60].

14.14.1 Dry Rubber Products

Tyres and related products: Pneumatic tyre manufacturing dominates the rubber industry in terms of the quantity of raw rubber consumption. About 50–60% of the rubber produced in the world is used for manufacturing tyres and related items. Tyre products include pneumatic tyres and tubes, solid tyres, tyre flaps, retread material, and puncture repair kits (PRKs). NR is the ideal base material for tyres for aircraft, racing cars, heavy duty vehicles such as trucks and buses, off the road vehicles, and tractors. An oil extended form of natural rubber could be used as base material for the manufacture of winter tyres [61].

Non-tyre automotive components: Non-tyre automotive applications of rubber are greater in number, weight, and cost than are tyre applications.

In a car there are about 750 rubber parts; roughly 375–400 chassis parts, and 350–375 body parts [62]. Many automotive applications have parallels in aerospace and rail transport.

Rubber products used in engineering, medical, sport, and household applications: Natural rubber is a very versatile material that has been successfully used in engineering applications for over a century. Anti-vibration mountings, flexible couplings, bridge bearings, rail pads, power transmission and conveyer belts, rubber covered rollers, cable covering, hoses, tubing, tank liners, extruded products, and components for domestic and industrial machinery are some of the industrial and engineering rubber products. Medical applications of natural rubber include dialysis tubing, hypodermic syringe seals, and baby dummies. Fabric coatings for inflatable boats, balloons, and footballs are examples of the applications of natural rubber in sports and recreational activities. Flooring, gloves, adhesive for pressure sensitive tapes, door/barrier mats, and footwear are familiar examples of household rubber products.

14.14.2 Latex products

About 10% of NR is marketed as latex concentrate, which is the base material for products manufactured directly from latex. The common techniques for manufacturing useful products from natural rubber latex are dipping, casting/moulding, foaming, extrusion, and coating [63]. A wide range of rubber products are made by latex dipping. The most significant of these are condoms, and various types of gloves, e.g. surgical gloves, household gloves, electrician gloves, and heavy industrial gloves [64]. Hollow castings and replicas can be manufactured by various types of moulding/casting techniques [65]. Natural rubber latex foam is an excellent cushioning material. The most important applications of foam are in mattresses, pillows, and seating in all kinds of vehicles [66]. Latex is also used as a binder for nonwoven fabrics, and as carpet backing. Elastic thread manufactured from natural rubber latex is an indispensable component of many types of garments. Natural rubber latex cement formulations are used in civil engineering applications.

14.15 Natural Rubber, a Green Commodity

In recent years there has been a growing awareness about global warming and its possible consequences. This naturally leads to the search for materials and processes which are environmentally friendly. While rubber products are essential to maintain the present quality of human life, a major part of the elastomer requirement is presently met by synthetic materials. In this context, an appreciation of the green credentials of natural rubber vis-à-vis its synthetic counterparts is highly desirable.

The energy input, other than solar energy, for NR production is only 15–16 GJ per tonne, while it ranges from 108–174 in the case of synthetic rubbers [67].

It is not only that synthetic rubber production is more energy-demanding, but it releases large amounts of CO_2 while NR production leads to removal of large quantities of CO_2 from the atmosphere and release of oxygen. Apart from rubber, a rubber plantation yields large volumes of biomass, part of which can be used as timber and the rest as wood for fuel. In fact, the rate of biomass accumulation in rubber trees is higher than that of many forest trees. Additionally, the annual waste produced by a rubber plantation amounts to around 7 tonnes per ha, and the rate of waste disintegration is faster than that of many other tree species [68]. Hence, nutrient recycling in rubber plantation is very efficient, leading to a minimum requirement of added nutrients in rubber cultivation. Every cultural operation in a rubber plantation, from land preparation to replanting, aids in improving the soil conditions. Even degraded soils can be made productive by a cycle of rubber cultivation. Thus, a mature rubber plantation is a dynamic and self-supporting ecosystem and a renewable source of rubber and biomass with minimum external agronomic inputs [69]. Moreover, rubber plantations provide a livelihood to over ten million small and marginal farmers in developing Asian and African countries. Also, it can be observed that rubber cultivation is gender-friendly and provides employment to a large number of women in these countries. These aspects of NR production have very important social implications.

14.16 Conclusions

Natural rubber is a biopolymer formed by linking thousands of isoprene units together. The organised production of natural rubber to meet growing industrial demand is just over one hundred years old. This biopolymer influences the quality of human life in many ways through the several thousands of products made from it, besides providing a livelihood to over ten million farmers, mostly in the developing countries of Asia and Africa. Natural rubber's plant origin, minimum energy requirement other than sunlight for production, and unique cultivation practices involving minimum use of agricultural inputs, makes it widely recognized as a green commodity. About sixty percent of the natural rubber produced in the world is used for the production of tyres and related items. With ever-increasing demand for more production and a better price, the future of natural rubber appears very bright.

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15

Electronic Structures and Conduction Properties of Biopolymers

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Abstract

The investigation of the electronic structure and conduction properties of biopolymers such as proteins and DNA has always been a challenging task due to the complexity of these systems. Both proteins and DNA have large molecules as their units arranged in aperiodic sequences. Proteins are composed of polypeptide chains made up from 20 different amino acid residues. Parts of the lengths of these polypeptides may assume random arrangements or form regular structures (such as, α-helical and β-pleated configurations) pertaining to their secondary structures and folding in space. DNA, on the other hand, has a double helix structure. Although the sugar-phosphate units forming the backbone of the helix are arranged periodically, the four nucleotide bases have an aperiodic sequence. Several ions and water molecules surrounding these biopolymers under biological conditions further add to the complexity of the calculations. In this chapter a detailed review of the progress achieved in the investigations on proteins and DNA by experimentalists and theoreticians is presented. Several experimental techniques and methodologies that have been used by different research groups are discussed. Finally, the scope of using artificial intelligence methods such as genetic and ant algorithms for theoretical modeling of biopolymers is investigated. It has been found that by using these computationally efficient methods, the electronic structure and conduction properties of biopolymers can be elucidated with much more ease in comparison to the conventional methods.

Keywords: Biopolymers, proteins, DNA, electronic structures, conduction properties, genetic algorithm, ant algorithm

15.1 Introduction

Over the past 50 years, spectacular advances have been made in X-ray diffraction, nuclear magnetic resonance spectroscopy, and several other techniques for determining structure. Theoretical studies of polymers have also been developing at a rapid pace with faster and more advanced computing capability. There is much interest at the moment in the possibility of using biological molecules such as proteins and DNA as electrical conductors. The possibility

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (437–458)

of electron delocalization over large regions and the ability of biomolecules to sustain electrical conductivity were first suggested by Szent-Gyorgyi [1], and this forms the theoretical basis of our understanding of charge carrier and excitation energy transfer observed in nucleic acids and proteins. The importance of the transport of separated electrons and holes or bound electron-hole pairs (excitons) in these macromolecules is obvious from the biochemical point of view. Furthermore, technologists are exploiting the electrical properties of the biopolymers like proteins and DNA in the field of molecular electronics. Thus, the investigation of their electronic structure and conduction properties offers great potential. Such investigations on proteins and DNA by experimentalists and theoreticians have previously been reviewed [2], and a further detailed discussion has been presented in this chapter. Furthermore, the use of artificial intelligence methods like genetic and ant algorithms for designing proteins with tailor-made conduction properties and studying the electronic structure of DNA with much more ease than conventional methods has also been discussed.

15.2 Electronic Conduction in Proteins

15.2.1 Introduction

The mechanism of energy transport in proteins is a fundamental problem of quantum biology. Biological systems are particularly challenging for *ab initio* quantum mechanical methods. The primary structure of a protein molecule is composed of a sequence of amino acid residues joined together by several peptide linkages which form the backbone of the macromolecule. Each peptide unit of a protein has a planar structure associated with the delocalized π-orbitals of the carbon and oxygen atoms together with the lone pair orbital of the nitrogen atom. The polypeptide sequences may assume regular geometries like the α-helix or the β-pleated sheet, as suggested by Pauling and coworkers [3] in 1951, giving rise to a secondary structure which may fold into a compact shape via loops and turns, hence defining the tertiary structure. Interactions between several chains of peptide bonds with the help of linkages like disulfide give way to the quaternary structure of the protein.

Although proteins isolated in their pure and dry state are poor conductors, the formation of charge-transfer complexes with electron-accepting molecules leads to conductivity sustained by positively charged electron "holes". Similarly, a charge-transfer interaction with an electron donor could result in the appearance of mobile electrons in the conduction energy levels of the protein. All important biological processes involve energy changes, and the energy requirement of the cell is fulfilled by hydrolysis of adenosine triphosphate (ATP) molecules. But in almost all biophysical processes, the site at the protein molecule where hydrolysis of ATP takes place is often separated by a large distance from the site where the energy is utilized. So the question arises, *"How is energy being transferred from one site to another at the cellular level?"* Szent Gyorgyi [1]

was the first to attempt an answer to the mechanism of energy transport in proteins, when he postulated that during biochemical processes, the transformations occurring at any part of the protein macromolecule are transferred to its other parts through electronic transport. The study of charge transfer in polypeptides has received a great deal of attention due to its relevance in biological activities and possible applications in molecular electronics. The charge transfer (CT) in biological systems has been the subject of interest for experimental as well as theoretical investigations, especially in the long-range charge transfer through peptide bridges and kinetic studies involving intramolecular CT reactions based on the potential energy surface (PES).

15.2.2 Investigations of Electronic Structure and Conduction Properties of Periodic and Aperiodic Polypeptides

Szent Gyorgyi's hypothesis on electronic conduction in biopolymers induced both theoreticians and experimentalists to carry out investigations on biopolymers. In 1946, he reported photoconductive effects for protein films containing dye molecules [4] and gave a new dimension to the concept of biological semi-conduction. A review of the various experimental and theoretical studies carried out on biomolecules such as proteins and DNA are presented.

15.2.2.1 Experimental Studies

The hypothesis on electronic conduction in biopolymers put forward by Szent Gyorgyi initiated many investigations of the electronic conduction properties of proteins [5–9]. These experiments which dealt with the calculation of d.c. conductivity with the formula $\sigma = \sigma_0 exp(-\Delta E/2KT)$ showed weak semi-conductivity in dark conditions. Later, Eley in 1963 [5] and Rosenberg in 1962 [10], carried out experiments on hydrated proteins and showed that conductivity increased exponentially with increasing hydration. While Rosenberg attributed this increase in conductivity to the decrease in energy barrier ΔE of the electronic transitions between the macromolecules, Eley concluded that the increase was due to the injection of electrons into the conduction bands of the protein molecules [11–14]. However one drawback of all these experiments was that the experimental results could not be interpreted correctly since native proteins with an unknown amount of inorganic and organic impurities had been used for investigations. Later on some transport measurements were carried out [15, 16] on these biopolymers, but the same was found true from these experiments as well. Burnel *et al.* [17] in their measurements on nucleosides and nucleotides indicated that the nucleotides exhibit lower semi-conduction activation energy than the nucleosides. This suggested that the phosphate group has a considerable effect on the semi-conductivity and may participate in charge transfer interactions to inject electron holes into energy states of the base pairs. Evidence for the same in the solid state was obtained from ESR measurements after gamma irradiation by Gregoli *et al.* in 1982 [18] and Graslund *et al.* in 1975 [19].

Apart from the above mentioned studies, other evidences exist in favour of electronic conduction in biopolymers such as: ESR studies by Gordy *et al.*in 1955 [20] and Henricksen *et al.* in 1963 [21], pulse radiolysis study by Adams *et al.*in1969 [22], flash photolysis study by Grossweiner and Usui in 1971 [23], studies on lysozyme, a study of the rate of hydration of cytochrome-c by electrons by Pecht and Faraggi in 1971 [24], and an examination of dielectric spectra of dopa-melanin to characterize electrical conduction mechanism and polarization effects by Jastrzebska *et al.* in 1998 [25]. The first measurement of electronic conduction effects in living tissue appears to be that obtained by Digby in 1965 and 1967 [26, 27] for the cuticle of various crustacea. In addition there is also evidence to suggest that electronic conduction properties of biopolymers may be involved in preventing the initiation of adverse effects on blood components and coagulation (Bruke, 1973, 1975) [28, 29].

15.2.2.2 Theoretical Investigations

On the basis of theoretical calculations, two pathways for electronic transport and delocalization in proteins were proposed initially. One was the hydrogen bonded network as suggested by Coulson, which runs perpendicular to both α-helical and β-pleated sheet structures and provides an extended conjugation pathway. The second was proposed by Brillouin [30] according to which, conduction in proteins takes place along the main polypeptide chain which contains several peptide linkages. In 1949, Evans and Gergely [31] performed the first energy band calculation for a model biopolymer (in tight binding approximation) based on the hydrogen-bonded network and proposed that electron delocalization occurs across the hydrogen bonds to give electronic coupling between all the peptide units in the hydrogen-bonded chains. Later in 1970, based on the original proposal by Brillouin [30] (that the side chain groups act as perturbations to produce impurity-type levels within the energy band gap), Fujita and Imamura [32] showed in their calculations of the electronic structure of α-helical polyglycine that there was considerable mixing in the σ and π orbitals of the energy bands and that σ and π electrons could not be treated separately. This conclusion was confirmed by Beveridge *et al.* [33] in their calculations on α-helical and β-pleated polyglycine using MINDO/2. In 1968, Snart [34] examined the d.c. electrical conductivity of solid gels of bovine plasma albumin under high vacuum to show a relationship between carcinogenic activity and molecular properties of carcinogens. Later in 1976, Szent Gyorgyi [35] also suggested the theory that there is a close relationship between conduction in proteins and cancer. According to him, an easy energy and charge transport in proteins and DNA is necessary for the normal functioning of the cell, and if this flow of energy and charge in these biopolymers is hindered it can lead to a cancerous state.

Although on the basis of earlier calculations, it had been widely accepted that electronic delocalization along the hydrogen bonds is realized in polypeptides; there were some experimental facts which seemed to contradict the

picture. ESR measurements show, for instance, that there is no evidence for an electron migration along the hydrogen bonds.

Eventually, Suhai [36] in his calculations took into account simultaneously both possibilities for delocalization. He calculated the all-valence electron energy band structure for β-polyglycine in the parallel-chain pleated sheet conformation, and investigated the anisotropy of the charge carrier effective masses and mobilities, and found the direction of the polypeptide backbone to be theoretically more favourable for electrical conduction than that of the hydrogen bonds. Kertesz *et al.*, in their studies [37] on electronic structure and transport properties of polyglycine showed that holes in the valence band are involved in conduction rather than electrons in the conduction band. In 1978 Kharkyanen *et al.* [38] made a very significant point that the energy band structure calculations using minimal atomic basis set were not sufficient, and there was a need to use better and extended basis sets to improve upon the results. From these results it follows that the most favorable mechanism of electron transport in proteins involves conduction along the main polypeptide chain rather than through the hydrogen bonded network.

Later on, the calculations were extended to aperiodic sequences by many research groups [39–46] in order to get an idea of the electronic and conduction properties of polypeptides. Some physical concepts such as proton transport in membrane pores and at protein-water interfaces, tunneling processes, and electronic conduction and induction effects in membrane-associated protein structures developed in the study of active transport of ions, in order to understand cellular processes at sub-molecular level, were described by Pethig [47] in his work in 1982. Suhai calculated the delocalized exciton states for α-helical polypeptide backbone, α-polyalanine (1985) [48], and electronic structure of a 2-D polyglycine network (1991) [49], first at *ab initio* level and then correcting the results for correlation. Studies have also been carried out on several macromolecules like pig insulin (investigation of electronic structures and hopping conductivity) [50–52], hen egg white lysozyme (by extended NFC method at *ab initio* level using minimal basis (MB) set and simulation of aqueous solution environment) [53], hagfish insulin [54], acidic PLA_2 from the venom of *A. halys pallas* (study of hopping centers important for biological activity of the enzyme [55]) to analyze the hopping conductivity and its influence on biological activity of the macromolecules. Ladik [56], on the basis of his detailed theoretical investigations showed that proteins are good disordered hopping conductors and their conductivity can be substantially influenced by binding of chemicals or by the effects of radiation. Koslowski [57] conducted studies of the electronic structure of the cytochrome subunit of the photoreaction center of bacterium *Rhodopseudomonas viridis* (1999), and an azurin mutant of the bacterium *Alcaligenes denitrificans* (2001) [58] (using tight-binding Hamiltonian) to characterize the eigenstates and eigenfunctions of the biopolymers. He concluded that the strongest degree of localization is observed close to the HOMO-LUMO gap (eigenstates become considerably more delocalized in the bulk of the valence and

the conduction band) and the presence of eigenstates on both sides of Fermi level reduces the HOMO-LUMO gap considerably.

The variation in the band gap of a $(Gly)_{30}$-helix with position in the peptide sequence has been investigated using AM1 semi-empirical molecular orbital (MO) theory within an extensive singles configuration interaction (CI) treatment by Herz *et al.* [59]. The calculations suggest that the band gap decreases from the C-terminus to the N-terminus for the model $(Gly)_{30}$-helix *in vacuo*. Guimarães *et al.* [60] carried out simulations of three biomolecules (thrombin, L-type calcium channel, and human *Cytomegalovirus protease*) through the application of simple explicit water models combined with protein force fields. Another significant calculation using semi-empirical methods was performed by Grigorenko *et al.* (2002) [61]. Ladik and his coworkers performed calculations on seven homopolypeptides, poly(gly), poly(ala), poly(ser), poly(thre), poly(leu), poly(ileu), and poly(val) [62] as well as acidic, basic, and sulfur containing homopolypeptides [63] using the inverse Dyson equation. In 2003, he [64] also computed band structures for homopolypeptides. On the basis of the band structures obtained, applying the intermediate exciton theory, he could calculate the UV spectra of different biopolymers and found the results for polyglycine and polyalanine to be in good agreement with the experiments. In 2003, Yu and Leitner [65] calculated the coefficient of thermal conductivity and diffusivity for myoglobin over a wide range of temperatures based on a calculation of the normal modes. In another study, Gray and Winkler [66] have established multi-step tunneling/hopping through the intervening amino acid side chains in the photosynthetic pathway which is two orders of magnitude faster than single-step electron tunneling in a re-modified copper protein. Another review addressing the anisotropic flow of energy in proteins is presented by Leitner [67].

In recent times, the ambit of theoretical investigations of proteins has been broadened with the use of artificial intelligence methods like the genetic (GA) and ant algorithms (AA). While GA [68–70] mimics the process of natural selection, AA [71–73] exploits the behaviour of real ants to iteratively search for an optimum solution to a hard combinatorial optimization problem. Thus, these techniques serve as powerful tools to solve the complex problem of designing model polypeptide chains with tailor-made conduction properties. As a step in this direction, our research group has combined these algorithms with NFC [40] and IIM [40, 74, 75] to elucidate the electronic properties (band gap and delocalization) of proteins. We have investigated model ternary aperiodic polypeptide chains (of 300 units) with polyglycine $(A)_x$, polyalanine $(B)_x$, and polyserine $(C)_x$ as the constituent units, where the overall conductivity of the resulting protein depends on the relative concentrations of A, B, and C. The band structure results obtained from *ab initio* Hartree-Fock crystal orbital method (using Clementi's minimal basis set) by Ladik *et al.* [40] were used as input for these studies. The results obtained from the two algorithms for the α-helical and β-pleated sheet configurations of the model polypeptide chains are shown in Table 15.1.

It can be seen that the results (Table 15.1) obtained from both GA and AA [76] are in good agreement. Thus, either of the algorithms can be used to replace

Table 15.1 Results obtained from GA and AA for the α-helical and β-pleated sheet configurations of polypeptide constituting polyglycine $(A)_x$, polyalanine $(B)_x$ and polyserine $(C)_x$ residues.

α-Helical Configuration			
Algorithm	**Optimum Percentage Composition**	**Band Gap (eV)**	**IPN**
GA	$A_1B_{96}C_3$	13.944	0.0053
AA	$A_1B_{98}C_1$	13.977	0.0051
β-pleated sheet configuration			
GA	$A_2B_1C_{97}$	14.721	0.0052
AA	$A_1B_1C_{98}$	14.751	0.0058

systematic search for obtaining the optimum solution (percentage compositions of the homopolypeptides which constitute the protein chain with minimum gap and maximum delocalization). For the α-helical configuration of the polypeptide (gly-ala-ser) the optimum solutions obtained from GA and AA ($A_1B_{96}C_3$ and $A_1B_{98}C_1$ respectively) depict that a higher percentage of alanine residue as compared to glycine and serine gives the most conducting protein chain. On the other hand, for the β-pleated configuration of the same protein system it was found that a higher percentage of serine compared to glycine and alanine would give a highly conducting protein chain with a low band gap and high delocalization (Table 15.1).

The electronic properties obtained were verified with the help of DOS (Density of States) curves (Figures 15.1a, 15.1b, 15.2a, 15.2b) corresponding to the optimum solutions. Since similar results were obtained from both GA and AA, the resultant DOS curves were also found to be alike. The DOS for the model polypeptide in α-helical configuration (Figures 15.1a, 15.2a) show sharp regions of allowed energy states which are mostly localized in the region from -11.5 eV to -12.2 eV and from 2.5 eV to 4.0 eV. From the DOS, the band gap for this system is expected to be around 13-14 eV which matches with our obtained band gap value. The DOS distribution for the same system in β-pleated sheet conjugation (Figures 15.1b, 15.2b) extends from -11.0 eV to -13.0 eV and from 3.5 eV to 5.2 eV and consists of broader regions as compared to the α-helical configuration. Again it can be seen that the obtained band gap value (approx. 14.7 eV) falls within the range (14 to 15 eV) of the gap value expected from the DOS curves.

The above results were further confirmed by systematic research and it was seen that the algorithms have proven to be efficient in saving computational time and effort without compromising upon accuracy. The information gained from various theoretical investigations can prove helpful to the experimental chemists in exploring the conduction behavior of complex biomolecules.

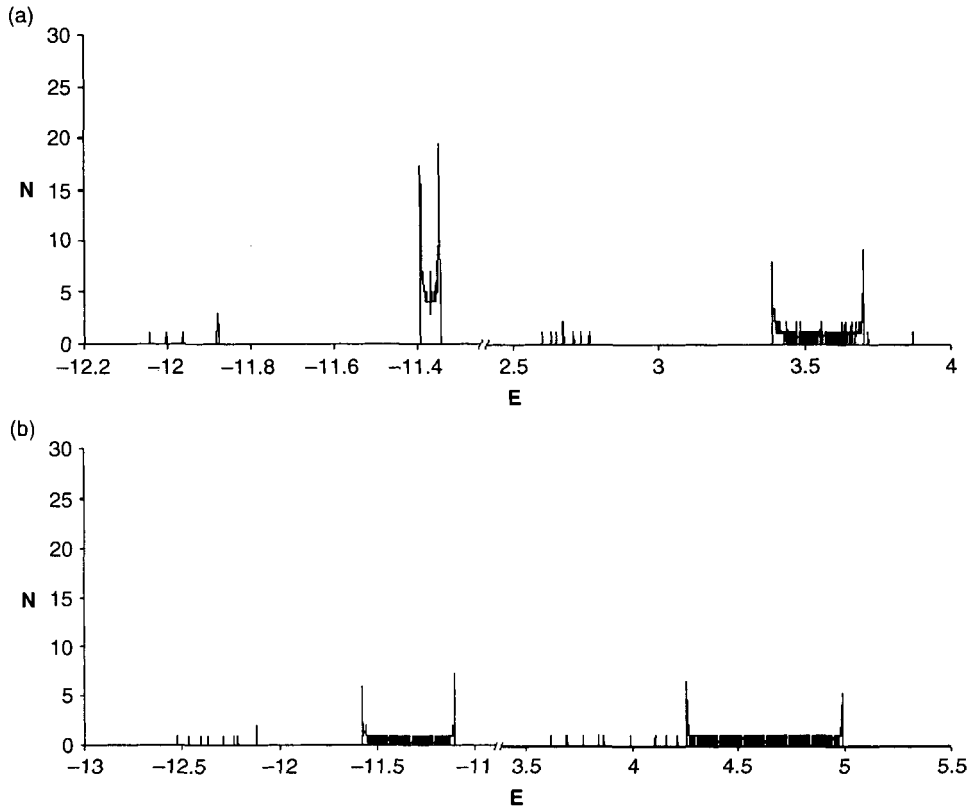

Figure 15.1 DOS plots corresponding to optimum GA compositions of aperiodic poly (gly-ala-ser) in (a) α-helical conformation and (b) β-pleated sheet conformation.

15.2.3 Factors Affecting the Conduction Properties of Proteins

Since biopolymers like proteins are present in our body in an aqueous medium surrounded by clusters of ions, the electronic structure calculations on these biomolecules need investigation in the presence of solvation shells. In other words, the effects due to the environment on the properties of the macromolecules also need consideration.

15.2.3.1 Effect of Solvent Molecules

There have been several studies for the determination of the water/solvent structure around biomolecules using the Monte Carlo method [77–84]. The band structures of several homopolypeptides have been calculated using the effective field of water molecules. In their calculations, Clementi *et al.* (1977) [77] represented each water molecule by 23 point charges contributing to the

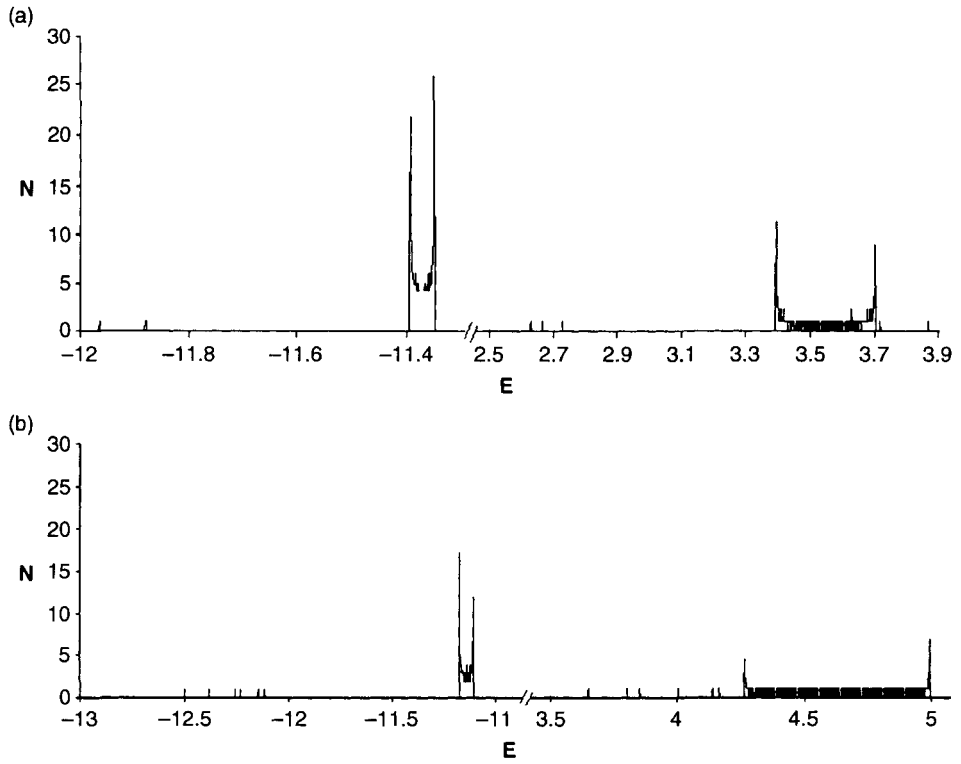

Figure 15.2 DOS plots corresponding to optimum AA compositions of aperiodic poly (gly-ala-ser) in (a) α-helical conformation and (b) β-pleated sheet conformation.

one-electron matrix in the crystal orbital calculations. To investigate the effect of hydration on proteins using the genetic algorithm, we have used the *ab initio* results for simulated structures (as reported by Liegener [45]) obtained by Monte-Carlo calculations. For model aperiodic polypeptide chains consisting of glycine and alanine residues, it was observed [85] that due to the effect of surrounding water molecules, the band gap of the β-pleated chain decreased by 1.364 eV (from 14.322 eV in absence of water to 12.958 eV in its presence) [85]. A large downward shift in the conduction band (CB) was observed due to the water point charges. Hydration has a strong influence on the width of the DOS curves (DOS plots within ref. 85). This is obviously due to the reason that the different chemical nature of the side chain (-H in glycine and $-CH_3$ in alanine) leads to an altogether different arrangement of the surrounding water molecules in their vicinity, which has cconsiderable effects on the corresponding energy bands.

Apart from the above mentioned methods, there are other possible approaches for simulating the solvent structure. For example, in the supermolecule calculations reported by Pullmann *et al.* (1975) [86], the approach

used was the theory of dielectric relaxation of biological water. In this theory suggested by Nandi and Bagchi [87] the time dependent relaxation of biological water is described in terms of a dynamic equilibrium between the free and bound water molecules. They assumed that only the free water molecules undergo orientation motion; the bound water contribution enters only through the rotation of the biomolecules. In all the approaches, it is a general observation that in the presence of water/solvent molecules the intrinsic conduction properties of the polypeptides increase due to the lowering of the band gap.

15.2.3.2 Effect of Ions in the Vicinity

Metal ions play a vital role in the biophysical world because they are precursors of several key enzymatic pathways, besides being participants in important biological processes like gene expression and formation of metallo-protein complexes. The ions present in the environment may influence the conduction properties of biopolymers through side chain reaction and through binding to these biopolymers. Dunicc *et al.* [88] reported strong effects of ions on the physical properties and chemical reactivity of biomolecules in the case of cell membranes in as early as 1979. Experimental evidence was provided by spectral studies performed by Balasubramanian and Shaikh [89] to support the proposition that salts affect the properties of proteins by binding/complexing to the peptide chain. Leigner *et al.* [84] performed calculations for band structures of polymers using the Monte-Carlo simulation method while considering the effect of sodium ions in the vicinity of the chains.

15.2.3.3 Effect of Side Chain Reactions

The amino acid residues in the protein chains have a tendency to produce their zwitter-ionic form in the presence of polar solvents, and may therefore develop charged side groups at physiological pH. These charged side groups may introduce new donor/acceptor levels, thereby considerably modifying the electronic structure and conduction properties of proteins. The first attempt to investigate the disorder effect of various side groups on the electronic structure of a protein was made by Laki *et al.* (90). The effect of side-chain protonation and tautomerisation of histidine on the electronic structure of proteins was investigated by Bakhshi and Ladik [91, 92] in 1986. An *ab initio* MB set calculation by Mezey *et al.* [93] on various neutral and protonated forms of amino acid models (serine, lysine, arginine, histidine and tyrosine) indicated that in proteins the arginine side chain is the most likely target for protonation. Some other studies [15, 16] show that keto-aldehydes, such as methyl glyoxal, when incorporated into the structure of protein molecules, can act as electron acceptors to produce mobile holes in the valence band.

15.3 Electronic Conduction in DNA

15.3.1 Introduction

DNA has long been known for its role as a carrier of genetic information. However, its electronic properties have attracted much interest in the past decade because of wide ranging applications, from nanoelectronic circuit technology [94–100] to long-range detection of DNA damage [101–102]. The self-assembly property of DNA, highly specific binding between its strands and the ability to synthesize in any particular sequence have led to a new approach to molecular electronics. Many scientists have given evidence of metallic conduction in DNA [103]. Thus, DNA could be useful in nanotechnology and this could overcome the limitations of silicon-based electronics [104]. These potential applications have led to numerous experimental as well as theoretical studies of the electronic structure and conduction properties of DNA. However, further work is required in this area to resolve many differences in both the theoretical and experimental results.

The structure of DNA discovered by Watson and Crick is illustrated by a right handed double helix, also known as the B form. The helix makes a turn every 3.4 nm, and the distance between two neighboring base pairs is 0.34 nm. Hence, there are about 10 pairs per helical turn. Each spiral strand is composed of a hydrophilic sugar phosphate backbone, and to each sugar unit is attached a hydrophobic base unit of adenine, guanine, cytosine, and thymine. The bases form complimentary pairs and bind the two strands together by hydrogen bonds between them [105, 106]. The unique molecular structure of DNA allows it to fulfill important biological tasks including coding, storage, and propagation of genetic information.

Prior to realizing the various applications, the electronic properties of the DNA bases need to be fully investigated. A major problem which arises in such investigations is of the treatment of aperiodicity of the DNA base stacks, making direct Self Consistent Field (SCF) calculations of their electronic structure impossible. Hence approximate methods need to be employed.

15.3.2 Mechanisms of Electron Transfer in DNA

It is generally believed that the bases in the centre of the double helix molecule form the pathway for electron transfer. The delocalized p-orbitals in consecutive bases overlap to form a channel for the movement of electrons [7]. The works of Ladik *et al.* [40] and Bakhshi *et al.* [41] have led to the assumption that the transmission channels are along the long axis of the DNA molecule [101, 107–109]. Several mechanisms have been given for charge transport in DNA, but the dominant ones are the coherent transport via extended molecular orbitals, and the thermal hopping mechanism.

15.3.2.1 Coherent Transport Mechanism

In this case, there is a single step electron tunneling through the potential barrier formed by certain base pairs [110]. This leads to an exponential decay of transmission with distance between the donor and the acceptor. The process is said to be coherent because the electron does not exchange any energy with the molecule during the transfer and the electron is never localized.

15.3.2.2 Thermal Hopping (TH) Mechanism

For long distance electron transfer, thermal hopping or the incoherent mechanism dominates. In this case, the electron is localized on the molecule and moves in a series of steps. Evidence for this mechanism in DNA duplexes comes from the experiments of Nakatani *et al.* [111].

15.3.3 Factors Affecting the Conductivity of DNA

The conducting behavior of DNA is governed by a variety of factors:

1. DNA sequence: In an aperiodic sequence the disorder along the one-dimensional chain keeps changing the environments of the various bases, hence their energy positions are scattered over a much wider range of energy, thus affecting the overall band gap of the resultant DNA molecule.
2. Length of the DNA chain: For chains of small lengths the electronic properties depend on the length of the chain, but for sufficiently large chains the conductivity of the DNA molecule is independent of the length of the chain.
3. Water and counter ions: Since DNA is a charged molecule the presence of water and counter ions in its environment influences its structure and conduction behaviour. The A-DNA structure is obtained with 5–10 water molecules present per base, while for 13 water molecules per base the B structure results.
4. Methods of preparation and detection.

15.3.4 Investigation of the Electronic Structure of DNA Base Stacks

15.3.4.1 Experimental History

Despite the current upsurge of interest about the conduction properties of DNA [105, 112], the subject is far from new. Eley and Spivey (1962) were the first to suggest that DNA could act as a conductor [77]. The experimental outcomes about the conductivity of DNA and its mechanisms vary from the wide gap insulating behaviour to the proximity induced superconducting one [95–98, 102, 105, 113–119]. A clear understanding of the nature of electronic conduction

in DNA is important to the study of biological processes like mutations and the repair of damaged bonds in cells. Despite intensive investigations, the mechanism of charge transfer in DNA remains a subject of controversy. Some studies show short range, whereas, others indicate long range charge migration [120–122], thus suggesting that the DNA duplex behaves like a one-dimensional molecular wire.

Eley and Spivey in 1962 proposed that the π-π interactions between stacked base pairs could lead to extended states with a reduced DNA energy gap and this could result in metallic DNA. From ESR measurements after gamma irradiation, Gregoli *et al.* [123] obtained evidence for electron transfer along the bases of DNA in a solid state. A detailed study of hole transfer reactions in DNA has been carried out by Giese *et al.* [124]. Scanning tunneling microscopy (STM) studies have shown that DNA molecules or its bases can self-assemble into various structures on different substrates [125-136]. Recently, Ohshiro *et al.* [137] reported STM images in solution that showed facilitated electron tunneling between a DNA base and a complementary base-modified STM tip. In 2007, Xu and Endres [138] demonstrated that DNA bases show base specific electronic signatures.

Fink and C. Schönenberger (1999) made the first direct electrical measurements for a single DNA rope, by using a modified high vacuum-low energy electron microscope [95], and concluded that the charge transport mechanism must be electronic in nature. This confirmed earlier experiments by Okahata *et al.* [139] who measured a finite conductivity for stretched films of DNA. In many published works, [96, 102,140–143] the d.c. current–voltage (I-V) data of double-stranded DNA molecules shows a characteristic symmetrical s-shaped curve with a non-conducting region near zero voltage, indicating that DNA behaves as a semiconductor. Kasumov *et al.* reported that DNA also behaves like a proximity-induced superconductor [98].

Some experimental results show that the conductivity of DNA can be increased by coating it with metals. Using the self-assembly property of DNA, Braun *et al.* [113] synthesized a DNA molecule between two electrodes and used it as a template for the deposition of silver to create a 100-nm wide conductive wire. It was further shown by Aich and coworkers [144] that DNA with zinc atoms between its bases also acts as a conductor. Keren *et al.* [145] have demonstrated sequence specific molecular lithography on substrate DNA molecules by harnessing homologous recombination by RecA protein. This constitutes an important step toward integrated DNA electronics. Distance dependence of charge transfer in DNA has been demonstrated by many experiments [146, 147], which leads to the suggestion that DNA could act as a "molecular wire". Hall *et al.* [122] have shown photo-induced oxidation by a rhodium metallointercalator in DNA and Dandliker *et al.* [148] demonstrated that this could be used to heal the thymine dimer (a DNA defect). Berlin and coworkers [149] considered mechanisms of hole injection and transport in stacks of Watson–Crick base pairs. Seeman and coworkers (150,151) have created topological structures with DNA. Self-assembled networks of DNA can be used to make transistor devices and, in fact, the first DNA field-effect transistor has already

been constructed [6, 95]. Recently, Shapir *et al.* have resolved the electronic structure of single DNA by transverse scanning tunneling spectroscopy [152]. They have determined the main groups of energy levels and the energy gap in the electronic structure of novel poly(G)-poly(C) DNA molecules adsorbed on a metal surface.

The experimental results from different groups vary widely and there is a lack of a consistent picture. Hence, it is difficult to draw firm conclusions about the transport of electrical charge through DNA.

15.3.4.2 Theoretical Efforts

The wide ranging experimental results on the conductivity of DNA have motivated theoretical efforts in this direction in order to come out with a clearer picture about the remarkable behaviour of the "molecule of life". The common theoretical approaches include *ab initio* studies and model calculations. The quantum master equations of the reduced density matrix [153] and the single step transfer theory of Marcus [154–157] govern the model calculations. According to Marcus, quantum-mechanical tunneling can occur when vibrational fluctuations bring the donor and acceptor states of the molecule into resonance.

A number of research groups have tried to use the *ab initio* methods to investigate in detail the electronic structure of DNA and the various factors affecting it. Lewis *et al.* [158, 159] performed the first band-structure calculation on B-DNA without solvent. By extending the use of Bloch's theorem to helical systems, Zhang *et al.* calculated the electronic structure of homogeneous DNA [160]. *Ab initio* methods have been used by various groups to study the effects of solvent, counter ions, and DNA dynamics [110, 161, 162]. In 2002, Gervasio *et al.* suggested that DNA can be doped by divalent or trivalent counter ions [162] and for a proper understanding of its electronic properties it is necessary to include solvation effects. These suggestions were further supported by other studies [163, 164] which concluded that the dynamics of the counter ions have a critical influence on charge migration in DNA.

Accurate *ab initio* calculations were carried out by Olofsson and Larsson [165] to theoretically decide about the transport mechanism of electrons in DNA. Effects of static and dynamic structural fluctuations on the hole mobility in DNA were considered by Grozema *et al.* [166], whereas the dependence of DNA-mediated conduction on the sequence of base pairs was studied by Roche [109]. Voityuk and his coworkers investigated the hole transfer through p-stacks containing chemically modified nucleobases [167, 168]. Carpena *et al.* [169] demonstrated that quasi random sequences of DNA may have longer-range correlations which can change their localization properties. Thus, one dimensional aperiodic DNA can behave as a conductor inspite of the disorder. Zhong [170] showed in 2003 that backbone disorder influences the electronic transport in DNA. Sekiguchi *et al.* [171] demonstrated that electron delocalization occurs along the backbone chains in the attosecond domain. Zwolak *et al.* [172] studied charge transport through nucleotides in the direction transverse

to the backbone axis, and found that each nucleotide has a unique electrical signature determined by the electronic and chemical properties of the bases. Hence, by exploiting the robustness of these signatures, electronic DNA sequencing may be possible. Using density functional theory Zikic *et al.* [173] found that the current through DNA bases is very sensitive to their geometrical conformation and orientation to the electrodes.

Very recently, Bende *et al.* [174] have applied the Hartree-Fock crystal orbital method in its LCAO (linear combination of atomic orbitals) form to determine the band structure of histone proteins by taking $0.041e$ charge transfer per nucleotide base from the PO_4- groups of poly(guanilic acid) to the arginine and lysine side chains in histones. The electronic structure of single stranded DNA base stacks has been studied by Kishor *et al.* in 2003 [175]. They have elucidated the effect of basis set and chain length on the electronic properties of DNA. Some of the results are summarized in Table 15.2.

The results clearly show that for chain lengths of 300 and 1000 units of aperiodic DNA, a small difference in the band gap is observed, whereas for periodic

Table 15.2 Calculated electronic properties-IP, EA and band gap (all values in eV) of periodic and aperiodic DNA base stacks of different chain lengths, for minimal and double zeta basis sets.

S.No.	Conformation of Sequence	No. of Units	IP	EA	Band Gap (eV)
Using Minimal Basis Set					
1	Periodic (A-T-G-C)	300	9.00	1.65	10.65
		1000	9.00	1.65	10.65
2	Periodic (A-G-C-T)	300	9.00	1.65	10.65
3	Aperiodic-1	300	8.80	1.50	10.30
		1000	8.75	1.45	10.20
4	Aperiodic-2	300	8.80	1.45	10.25
		1000	8.75	1.45	10.20
Using Double Zeta Basis Set					
1	Periodic (A-T-G-C)	300	8.00	2.45	10.45
		1000	8.00	2.45	10.45
2	Periodic (A-G-C-T)	300	8.00	2.50	10.50
3	Aperiodic-1	300	7.8	2.30	10.10
		1000	7.75	2.30	10.50
4	Aperiodic-2	300	7.75	2.35	10.10

DNA strands, the results obtained remain unaffected with the increase in chain length. Furthermore, the band gap decreases slightly with the use of a better basis set. This is because in a minimal (single zeta) basis set, only those orbitals that are occupied in the free atoms are considered, whereas in an extended basis set, each valence orbital is supplemented by a delocalized orbital of the same quantum number. Hence, the use of larger basis sets for theoretical calculations on DNA strands brings their band gaps closer to the actual *in vivo* values.

Despite the numerous experimental and theoretical investigations carried out to gain an insight into the conduction behaviour of DNA, its electronic properties continue to remain controversial. Understanding the conduction behaviour of a polyelectrolyte system like DNA is by itself a major scientific problem, with the aperiodicity of the base stacks further complicating it. To overcome this problem, we have attempted to use the ant algorithm (AA) (in combination with NFC and IIM) for exploring the electronic structure and conduction properties of DNA. Thus, the relative concentrations of the four nucleotide bases [Adenine(A), Cytosine(C), Guanine(G), and Thymine(T)] which would constitute the most conducting (with minimum HOMO-LUMO gap and optimum delocalization of electrons) DNA strand have been determined. The investigation has been done using the band structure results obtained by *ab initio* Hartree-Fock crystal orbital method using minimal basis set as input [176]. The optimum solution (concentrations of the four bases) obtained, the corresponding band gap and inverse participation number (IPN-a measure of electronic delocalization) are shown in Table 15.3. It can be seen from the results that the most conducting single DNA strand contains guanine in the highest percentage, closely followed by thymine, whereas adenine and cytosine are present in very little concentration. It was further observed that guanine has the lowest ionization potential in the band structure. The band gap obtained corresponds well with that expected (10-11 eV) from the DOS curves (Figure 15.3) for the system. These curves show that both the valence and conduction bands consist of sharp peaks with small gaps in between. For a strand of 300 units the bands are localized in the region -8.8 eV to 11.2 eV and from 1.4 eV to 2.8 eV.

Table 15.3 Calculated electronic properties of single stranded DNA and the optimum compositions of the bases Adenine (A), Cytosine (C), Guanine (G) and Thymine (T) obtained from AA using minimal basis set.

Optimized Solutions for Single Stranded Aperiodic DNA Base Stacks Using Minimal Basis Set	
No.of units	300
Optimum percentage composition	$A_4C_1G_{50}T_{45}$
Band gap (eV)	10.1936
IPN	0.2462

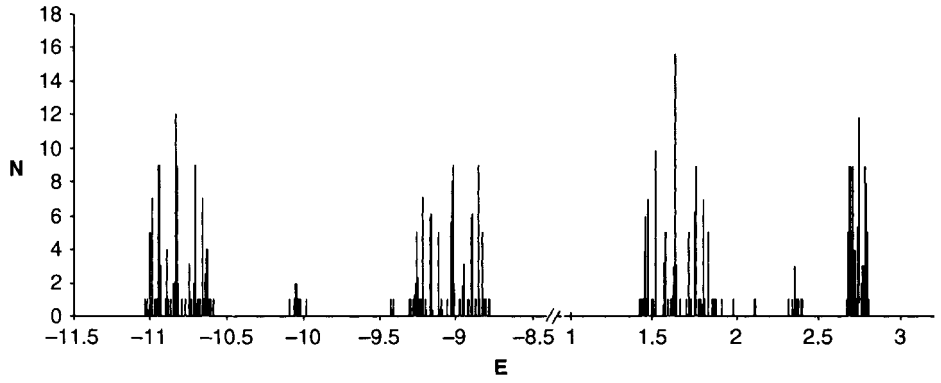

Figure 15.3 DOS plot corresponding to the optimized AA solution (from AA) for single stranded aperiodic DNA base stacks using minimal basis set.

It can be clearly seen from the results that the ant algorithm is quite useful in the investigation of the electronic structure and conduction properties of DNA, as it has the advantage of reaching the optimum solutions in much less time than systematic search. This algorithm can be further coupled with other machine learning techniques to produce better performing 'hybrid' algorithms [177, 178]. The results thus obtained can help experimentalists in probing the conduction behaviour of DNA without any waste of time and effort.

Such a wide variety of experimental and theoretical results have led to the conclusion that the DNA oligomer is a promising candidate for application as a molecular wire, and can thus serve as a building block in molecular electronics. One important example of a potential application of conducting DNA in molecular electronics is the DNA chip. It is used for DNA sequencing, disease screening, and gene expression analysis. Hence, it can be said that the "molecule of life" can create a technological revolution. The first step in this direction has already been taken with the creation of a DNA field-effect transistor [118], and more DNA based molecular electronic devices are likely to follow.

15.4 Conclusions

The present chapter provides a detailed description of the theoretical and experimental investigations on the electronic structure and conduction properties of biopolymers such as proteins and DNA. Novel and efficient methodologies such as the genetic and ant algorithms are presently being used to elucidate the electronic structure of these biopolymers, as well as to study the effect of their biological environment on their properties. The future will see further developments in such application based algorithms. These methods are computationally cost effective and can help researchers in designing biopolymers with pre-specified properties. It can be concluded from the results of a wide variety of studies presented here that both proteins and DNA can

act as electrical conductors, and thus have potential applications in the field of molecular electronics. Many such applications have already been realized and more are likely to follow in the near future.

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PART 4
BIOPOLYMERS FOR SPECIFIC APPLICATIONS

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (3–34)

16

Applications of Biopolymers in Agriculture with Special Reference to Role of Plant Derived Biopolymers in Crop Protection

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Abstract

Biopolymers are a diverse and remarkably versatile class of materials that are either produced by biological systems or synthesized from biological source materials. Biopolymer seed coating serves as soil stabilizer, seed protector, yield-enhancer, and plant growth regulator. Biopolymers dry quickly, dissolve rapidly in water, form effective water-soluble film, readily adhere to seeds, minimize the required dose of fungicides, and provide excellent control of plant diseases thereby contributing to enhanced plant productivity. Biopolymer seed coatings with various active ingredients precisely provide early access to each sown seed with nutrients, fertilizers, and pesticides in an accurate and timely manner with long lasting ingredients. New biocompatible and biodegradable biopolymers are produced from plants, microbes, animals, renewable agricultural wastes, and feedstocks. Modern biotechnology and nanotechnology tools are used to create new materials by genetic modification of an organism in order to produce greater quantities of a particular biopolymer. Biopolymer applications in agriculture are increasing, and there is an emphasis on further research for more expertise in biopolymer engineering and genetic modification of biological systems to produce designed biopolymers for agricultural use.

Keywords: Biopolymers, seed coating, precision agriculture, crop productivity

16.1 Introduction

Polymers, either natural or synthetic, have become an integral part of our daily life and also have a vital role in industry and the economy. Natural polymers like nucleic acids, proteins, polysaccharides, etc., have specific roles such as transportation, processing and manipulation of biological information, or and to act as fuel for cellular activity and to provide structural elements in living systems [1]. On the other hand, synthetic polymers like nylon, polyethylene, and polyurethane have penetrated our daily lives in such a way that they have become

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (461–482)

indispensable for smooth functioning. Application of synthetic polymers range from automobile bodies to packaging, compact discs to clothing, and food additives to medicine, cosmetics, water treatment chemicals, industrial plastics, absorbents, biosensors, and even data storage elements. These manmade polymers pervade virtually every aspect of modern society [2]. Synthetic polymers are not biodegradable, are difficult to dispose of and the synthesis of most of them involves the use of toxic compounds or generation of toxic byproducts. Therefore, these synthetic polymers have caused a concern from an environmental perspective and also from a health point of view, thereby necessitating the exploration of eco-friendly and safe alternatives [3]. One such alternative approach is the use of polymers which are of direct biological origin, or which are synthesized using biological precursors. The advent of modern biotechnology and genetic engineering tools have enabled synthesis and production of biopolymers. The applications of such biodegradable and eco-friendly biopolymers are far-reaching, ranging from agriculturally or bacterially derived thermoplastics that are truly biodegradable, to novel medical materials that are biocompatible, to water treatment compounds that prevent mineral buildup and corrosion. The scope and application of biopolymers is vast and versatile, finding a place in all sectors of human endeavor. They can be used as adhesives, absorbents, lubricants, soil conditioners, cosmetics, drug delivery vehicles, textiles, high-strength structural materials, and even computational switching devices [3, 4].

16.2 Biopolymers

The word 'biopolymers' is used to describe a variety of materials. In general, however, biopolymers fall into two principal categories: 1) polymers that are produced by biological systems such as microorganisms, plants, and animals; and 2) polymers that are synthesized chemically but are derived from biological starting materials such as amino acids, sugars, natural fats, or oils [2]. Biological polymer or biopolymers are macromolecules derived or produced from natural sources and living organisms. Cellulose, starch, chitin, proteins, peptides, DNA, and RNA are all examples of biopolymers in which the monomeric units, respectively, are sugars, amino acids, and nucleotides [5, 6].

A major but defining difference between polymers and biopolymers can be found in their structures. Polymers, including biopolymers, are made of repetitive units called monomers. Biopolymers often have a well-defined structure, though this is not a defining characteristic (e.g. lignocellulose). The exact chemical composition and the sequence in which these units are arranged is called the primary structure in the case of proteins. Many biopolymers spontaneously fold into characteristic compact shapes which determine their biological functions and depend in a complicated way on their primary structures. In contrast, most synthetic polymers have much simpler and more random (or stochastic) structures [7, 8].

Biopolymers are renewable, because they are made from natural materials which can be grown year to year indefinitely. These natural materials come

from agricultural nonfood crops, microbes, animals, etc. Therefore, the use of biopolymers would create a sustainable industry. In contrast, the feedstocks for polymers derived from petrochemicals will eventually run out. In addition, biopolymers have the potential to cut carbon emissions and reduce CO_2 quantities in the atmosphere. This is because the CO_2 released when they degrade can be reabsorbed by crops grown to replace them, making them close to carbon neutral [9]. Some biopolymers are biodegradable and they are broken down into CO_2 and water by microorganisms. In addition, some of these biodegradable biopolymers are compostable and can be put into an industrial composting process and will break down by 90% within six months [9].

16.3 Sources of Biopolymers

Novel families of biopolymers can be produced in significant quantities from lower and higher plants, microbes and animals, and also the readily available renewable agricultural waste and feedstocks. Naturally derived biopolymers are biocompatible, biodegradable, and versatile, permeating all fields of agriculture and offering positive attributes in terms of green chemistry. Biopolymers are being produced from a variety of raw materials, many of which reflect the diversity of indigenous plants and the region's local land and sea harvest. These include edible roots, fruit and vegetable fibers, the ubiquitous palm tree, and the remains of shellfish.

16.3.1 Plants

Cereal grains, some species of grasses, legumes, oil seed plants, gum and resin yielding shrubs/trees, etc., are rich sources of biological polymers. Cereals are agricultural raw materials very rich in biopolymers such as starch, proteins, polysaccharides, and lipids. Cereal grains like maize, sorghum, and rice are also a rich source of biopolymers [10]. Individual biopolymers are separated and purified from cereals through dry or wet milling. In decreasing order of quantity, the biopolymers of cereals grains are: Starch (approx. 70%) which has two types of molecules, amylopectin (branched chained) and amylose (essentially straight chained); proteins of various types (approx. 10–12%), the aqueous alcohol soluble prolamin group accounting for some 60% of the protein in most cereal grains (it should be noted, however, that prolamins themselves are a very varied group of proteins, with widely differing functional properties); non-starch polysaccharides (dietary fibre) (approx. 9–12%) of various types, including cellulose, mixed linkage beta-glucans, and pentosans (mainly xylans); and lipids (approx. 3–5%), mainly triacylglycerols (triglycerides) [11]. Biopolymers can be sourced from oilseed crops like *Arabidopsis thaliana*, corn, rapeseed/canola, soybean, and other crops such as cotton (in fiber), potato, tobacco, and switchgrass [12]. The aliphatic biopolymer cutan, previously only known to have come from *Agave americana* and *Clivia miniata*, was isolated in high purity from the leaf cuticles of several drought-adapted plants having

succulent leaves with thick cuticles [13]. Meanwhile in Venezuela, the yuca root, an edible root also known as mandioca or cassava, is being tapped for a starch that can be fermented and extruded into biopolymer pellets. At the Center of Research in Polymer Processing (CIPP) in Colombia, work is underway to develop natural-fiber reinforcements. Materials under study include fibers extracted from pineapples, rice and rose stems, and palms.

Many plants, when damaged, exude resinous materials as a defence mechanism. For example, the shrub *Commiphora abyssinica* produces a resin which contains a number of antibacterial and antifungal compounds. The role of the resin is to seal the wound and prevent bacteria and fungi from entering and damaging the plant. Rosin is produced as a physical barrier to infectious organisms by pine trees when the bark is damaged. Similarly, rubber is a defensive secretion. Gum biopolymers are harvested from wounded plants, extracted from seeds, and the wood of various plants/trees belonging to different families (Table 16.1). Gums are mainly the exudates of material in response to injury or wounding, which are exudated due to the breakdown of compounds in injured cells. Primarily gum exudates act as a seal for wounds and protect the plant against invasion by pathogens. Chemical constituents of gums are polysaccharides such as salts and sugars, arabinose, fucose, galactose, mannose, rhamnose, and xylose. The calcium, magnesium, and potassium ions of the salts cause the gum molecules to associate with water. Different types of biopolymer gums are exuded by a diverse array of plants/trees: e.g. gums are from wounded trees of *Acacia Arabica, Acacia senegal, Astragalus gummifer, Astragalus* spp., *Moringa oleifera, Carica papaya; Sterculia urens* (Sterculiaceae) and *Anogeissus latifolia*. Some of the gums are extracted from the endosperm of seeds of some legume species or are extracted from the wood of *Ceratonia siliqua* and *Ceratonia siliqua*, and from wood chips from *Larix occidentalis*.

16.3.2 Microbes

A huge variety of biopolymers, such as polysaccharides, polyesters, and polyamides, are naturally produced by various microorganisms. Microbial biopolymers are produced by a range of microorganisms cultivated under various growth and nutrient conditions. These polymers, usually lipids, accumulate as storage materials (as mobile, amorphous, liquid granules) that accumulate as granules within the cytoplasm of cells, meant for microbial survival under stressful conditions. Biopolymers such as polyhydroxybutyrate (PHB) granules were first observed by Lemoigne in *Bacillus megaterium* (a gram positive bacterium) in 1926. Since then several polyhydroxyalkanoates (PHAs) have been shown to be elaborated on by some archaebacteria and several gram positive and gram negative bacteria. Some examples are *Ralstonia eutropha* and other *Ralstonia* spp., *Pseudomonas putida* and other *Pseudomonas* spp., *Bacillus mycoides, Alcanivorax borkumensi, Rhodococcus ruber,* etc. Several short and medium chain length PHAs are synthesized by bacteria like *Ralstonia eutropha* and *Pseudomonads* [14].

Table 16.1 List of gum/resin producing plants/shrubs/trees and the family to which they belong.

Sl. No.	Plant/Tree	Family
1.	*Acacia arabica*	Mimosaceae
2.	*Acacia catechu*	Mimosaceae
3.	*Acacia nilotica*	Mimosaceae
4.	*Acacia senegal*	Mimosaceae
5.	*Acacia jacquemontii*	Mimosaceae
6.	*Acacia leucophloea*	Mimosaceae
7.	*Anogeissus pendula*	Combretaceae
8.	*Anogeissus latifolia*	Combretaceae
9.	*Aegle marmelos*	Rutaceae
10.	*Astragalus gummifer*	Fabaceae
11.	*Azadirachta indica*	Meliaceae
12.	*Bauhinia racemosa*	Caesalpiniaceae
13.	*Boswellia serrata*	Burseraceae
14.	*Bombax ceiba*	Bombacaceae
15.	*Buchnania latifolia*	Anacardiaceae
16.	*Butea monosperma*	Fabaceae
17.	*Carica papaya*	Caricaceae
18.	*Ceratonia siliqua*	Fabaceae
19.	*Cochlospermum religiosum*	Bixaceae
20.	*Commiphora abyssinica*	Burseraceae
21.	*Commiphara wightii*	Burseraceae
22.	*Larix occidentalis*	Pinaceae
23.	*Leucaena leucocephala*	Mimosaceae
24.	*Lannea coromandelica*	Anacardiaceae
25.	*Moringa oleifera*	Cesalpiniaceae
26.	*Miliusa tomentosa*	Anonaceae
27.	*Prosopis cineraria*	Fabaceae
28.	*Prosopis julifera*	Fabaceae
29.	*Soymida febrifuga*	Meliaceae
30.	*Sterculia urens*	Sterculiaceae

Microbial systems have proven to be low-cost, environmentally safe methods for improved biopolymer production. *Leuconostoc mesenteroides*, *Pseudomonas pseudomallei*, and *Bacillus* spp., and biopolymers produced by microbes have received much attention due to their easy adaptability to tools of genetic engineering [15].

16.3.3 Animals

The most common biopolymers derived from animals are chitin and chitosan. Chitin is a macromolecule found in the shells of crabs, lobsters, shrimps, and insects. The primary unit in the chitin polymer is 2-deoxy-2-(acetylamino) glucose. Chitin can be degraded by chitinase. Chitosan is a modified natural carbohydrate polymer derived from deactylation of chitin, which occurs principally in animals of the phylum *Arthropoda*. Chitosan is also prepared from squid pens. Chitin is insoluble in its native form but chitosan, the partly deacetylated form, is water soluble. The materials are biocompatible and have antimicrobial activities as well as the ability to absorb heavy metal ions [16].

Chitosan is a plant defence booster and its agricultural applications are for stimulation of plant defence. The chitosan molecule triggers a defence response within the plant, leading to the formation of physical and chemical barriers against invading pathogens [16].

16.3.4 Agricultural Wastes

Agricultural wastes form a rich source of biopolymers. There is use for agricultural oils as an inexpensive carbon source to produce different kinds of biopolymers. *Acinetobacter venetianus* produced chemically and biologically distinct emulsan variants in culture on soy molasses and tallow oil. The important polymer emulsan can be synthesized from this inexpensive carbon source [17]. PHA (polyhydroxyalkanoate), a biodegradable linear polyester derived from bacteria in agricultural wastes, is having applications in agricultural film. To reduce production costs, the material is being extracted from byproducts of winemaking. The possibility of using xylan as an agricultural by-product for the production of composite films in combination with wheat gluten is being explored. Wheat gluten/xylan composite films having different characteristics can be produced depending on the xylan type, composition, and process conditions [18].

16.3.5 Fossils

Sediments of higher plant remains like seeds, pollen, cuticle, bark, and wood remains, indicated that their fossilization was comprised of highly resistant biopolymers like cutan, suberin, and lignin. In most cases only the seed coats are found in the geological record, because the outer layers of seeds contain resistant compounds to protect the genetic material against physical and chemical processes such as temperature and humidity changes and bacterial

and fungal attacks. Hence, seeds, and particularly their resistant layers, have a large potential of entering the geosphere and may become selectively enriched [19]. Chitin is present in fossil insects from the Oligocene (24.7 million years ago) lacustrine shales of Enspel, Germany [20]. Analysis of modern *Metasequoia* leaves revealed the presence of the structural polyester cutin, guaiacyl lignin units, and polysaccharides. *Metasequoia* fossils from the Eocene of Republic (Washington State, USA), showed a significant aliphatic component, fossils from the Eocene of Axel Heiberg Island revealed the presence of lignin and an aliphatic polymer up to C_{29} with cellulose, and fossils from the Miocene Clarkia deposit (Idaho, USA) revealed lignin and an aliphatic polymer up to C_{27} without any polysaccharides [21].

16.4 Application of biopolymers in agriculture

The economic losses in agricultural production worldwide are attributed to pests and pathogens that cause destructive diseases, thereby affecting seedling stands and yield [22]. Therefore, ensuring the quality of the seed will in turn protect the seedlings and plant stand from such adversaries. Seed treatment with various chemicals and with advanced technology has enabled the minimization of pests and diseases and as a result, the economic losses [23].

Since the introduction of plastic films in the 1930s and 1940s for greenhouse coverings and fumigation and mulching, agricultural applications of polymers have grown at an enormous rate. All principal classes of polymers, i.e. plastics, coatings, elastomers, fibres, and water-soluble polymers are presently utilized in applications which include the controlled release of pesticides and nutrients, soil conditioning, seed coatings, gel plantings, and plant protection. However, degradable plastics are also of interest as agricultural mulches and agricultural planting containers. Ultimate biodegradability, as in composting, is also of some interest as it would permit degradable plastics to be combined with other biodegradable materials and be converted into useful soil-improving materials.

Chitin and chitosan are naturally occurring compounds that have potential in agriculture with regard to controlling plant diseases. They were reported to be active against fungi, viruses, bacteria, and other pests. They have also been utilized to chelate nutrients and minerals, preventing pathogens from accessing them, or to enhance plant innate defences. Fragments from chitin and chitosan are known to have eliciting activities leading to a variety of defence responses in host plants in response to microbial infections, including the accumulation of phytoalexins, pathogen-related (PR) proteins and proteinase inhibitors, lignin synthesis, and callose formation. Based on these and other proprieties that help strengthen host plant defences, interest has been growing in using them in agricultural systems to reduce the negative impact of diseases on the yield and quality of crops [24]. Chitosan resulted in yield increases of nearly 20% in two out of three tomato trials. In all tomato trials, chitosan applications resulted in a significant improvement in powdery mildew disease control [25].

Biopolymer derived mulches help with plant growth and then photodegrade in the fields, thereby avoiding the cost of removal. These mulches improve the plant growth rate due to their ability to conserve moisture, reduce weeds, and increase soil temperatures. A two-to-threefold increase in melon yield was recorded using black polyethylene mulch. Elimination of weeds and avoidance of soil compaction by the use of mulch eliminates the need for cultivation, therefore root damage and stunting or killing of plants is further avoided. Fertilizer and water requirements are also reduced [26].

Starch-polyvinyl alcohol film consisting of up to 40% starch, urea, ammonia, and various portions of low-density polyethylene (LDPE) and poly(ethylene-co-acrylic acid) (EAA), could be coated with a thin layer of water-resistant biopolymer to give a degradable agricultural mulching film. The EAA acted as a compatibilizer, forming a complex between the starch and the PE in the presence of ammonia. The resulting blend could be cast or blown into films, and had physical properties approaching those of LDPE [26].

In addition, transparent polyethylene is more effective in trapping heat than black or smoke-grey films. Radiative heat loss at night, as the soil cools, is lessened by polymer films. In some cases, weed control has been reported because of solar heating of the polyethylene mulches [26]. If left in place, however, conventional films can cause problems during harvesting or during cultivating operations the next year. Removal and disposal are costly and inconvenient. Therefore, interest in the development of biodegradable or photodegradable films with short service lifetimes has grown. The plastics used for mulch films are generally low density polyethylenes, poly(vinyl chloride), polybutylene, or copolymers of ethylene with vinyl acetate. A particularly interesting photodegradable system consists of a mixture of ferric and nickel dibutyldithiocarbamates, the ratio of which is adjusted to provide protection for specific growing periods. The degradation is tuned so that when the growing season is over the plastic will begin to photodegrade. Another additive system being proposed for this application includes a combination of substituted benzophenones and titanium or zirconium chelates. The principal commercial degradable mulch is photodegradable poly (1-butene).

Polylactone and poly(vinyl alcohol) films are readily degraded by soil microorganisms, whereas the addition of iron or calcium accelerated the breakdown of polyethylene. Degradable mulches should break down into small brittle pieces which pass through harvesting machinery without difficulty, and do not interfere with subsequent planting. Effective fumigant mulches require reduced-porosity films which reduce the escape of volatile chemicals, i.e. nematocides, insecticides, herbicides, etc., and therefore allow for lower application rates.

Controlled release (CR) is a method by which biologically active chemicals are made available to a target species at a specified rate and for a predetermined time. The polymer serves primarily to control the rate of delivery, mobility, and period of effectiveness of the chemical component. The principal advantage of CR formulations is that less chemicals are used for a given time period, thus lowering the impact on non-target species and limiting leaching, volatilization, and degradation [27]. Controlled release polymeric systems can be divided into two broad categories. In the first, the active agent is dissolved,

dispersed, or encapsulated by the polymeric matrix or coating. Release generally occurs by diffusion processes or by the biological or chemical breakdown of the matrix. In the second category, polymers contain the active agent as part of the macromolecular backbone or pendent side chain. Release results from biological or chemical cleavage of the bond between the bioactive agents and the polymer [26, 27]. Starch, cellulose, chitin, alginic acid, and lignin are among the natural polymers used in CR systems. These have the advantage of being abundant, relatively inexpensive, and biodegradable [26].

16.5 Seed coating for value addition

Generally, pesticides are applied either by seed pelleting, seed dressing, or seed soaking with an adhesive material which is mixed throughout the coating material or can be added in discrete layers or in the outermost part of the pellet. It has now been found that biopolymers derived from biological sources can provide excellent water-soluble or water-dispersible film, are stable in storage and have good adherence to seed. A number of trees like *Acacia arabica, Moringa oleifera, Carica papaya* and *Azadirachta indica* are well known to produce gum exudates during their different stages of growth. An approach employing these benefits can minimize the recommended dose of fungicides by reducing dust-off, and provides excellent control of plant diseases. The gum biopolymers have particular application in the protection of seeds against fungal pathogens when combined with one or more fungicides. Biopolymers dry quickly, dissolve rapidly in water and do not inhibit germination [28].

A number of natural biopolymers containing pesticide active groups have been in the market for the purpose of developing ideal controlled release formulations for fungicides and herbicides. These biopolymers have been derived by natural exudation. Several factors appear to be important in governing the rates of release of active moieties. These factors include environmental conditions as well as the effects of the biopolymers' compositions, properties and chemical structures, and the simulated conditions necessary to prolong the activity under suboptimal conditions such as the pH and temperature.

Precision seed coating technology has become vital in the current global agriculture. Biopolymer seed coatings precisely provide each sown seed the nutrients required with accuracy; fertilizer seed coatings improve early access to nutrients by the seedlings, fungicide seed coatings reduce seedling diseases, and rhizobial preparations enhance nodulation of legumes. Coating seeds with macronutrients (i.e. phosphorus, calcium, sulphur), micronutrients (i.e. manganese, zinc, molybdenum), hydrophilic substances to attract water, peroxides to provide oxygen, and antidotes in the form of pre-emergence herbicides is widely accepted. Higher value seeds are subjected to biopolymer based fungicide coating which offers broad-spectrum protection against major soil-borne and seed-borne pathogens [29].

The seed coating effect of the biopolymer zein, extracted from the gluten meal of maize, was tested on the germination rate of sugar beet (*Beta vulgaris* L.) and broccolis (*Brassica oleraceae* var *italic* L.) seeds under laboratory conditions,

and the resultant coating elevated the hydrophobic feature due to the high content amino acids present in the structure of the zein, causing an overall delay in sprouting and germination [30].

The biopolymeric coating has several advantages and its potential benefits include prolongation of active ingredients activity. Biopolymer coating allows much lower amounts of effective ingredient than conventional pesticides to be used because it releases the required amount of active agent over a longer period, reducing the number of applications through achieving a lengthier duration of activity by a single application, thereby reducing cost. Biopolymer coating eliminates the time and cost of often repeated applications because less active materials are needed, thereby reducing toxicity, environmental pollution, and the degradation of pesticides [31, 32].

Maize seeds primed with chitosan had no significant effect on germination under low temperatures, but enhanced germination index, reduced the mean germination time, and increased shoot height, root length, and shoot and root dry weights in two tested maize lines [33]. In both tested lines, chitosan induced a decline in malonyldialdehyde content, altered the relative permeability of the plasma membrane, and increased the concentrations of soluble sugars and proline, and of peroxidase and catalase activities. In other studies, seed priming with chitosan improved the vigor of maize seedlings [34]. It was also reported to increase wheat seed resistance to certain diseases and improve their quality and/or their ability to germinate [35]. Similarly, peanut seeds soaked in chitosan were reported to exhibit an increased rate of germination and energy, lipase activity, and gibberellic acid and indole acetic acid levels [36]. Ruan and Xue [37] showed that rice seed coating with chitosan may accelerate their germination and improve their tolerance to stress conditions. In carrot, seed coating helps restrain further development of *Sclerotinia* rot [38]. Chitosan has also been extensively utilized as a seed treatment to control *F. oxysporum* in many host species [39].

16.6 Plant Derived Biopolymers in Plant Growth Promotion

Plant-based exudates are excellent gum biopolymers which contain plant growth-regulating hormones with priming potential without any side effects. Gums and resins have been largely used as carrier or adhesive in various biocontrol and chemical formulations. In terms of actual sales, agricultural end uses constitute the largest and most successful market for chitin and chitosan polymers. The fungi resistant properties of chitosan have resulted in its application as a fertilizer, soil stabilizer, and seed protector. It is a yield-enhancing agent for wheat, barley, oats, peas, beans, and soybeans. Thus, chitosan is used both as a seed coating and as a plant growth regulator. Plant derived dextrans are used extensively in oil drilling muds to improve the ease and efficiency of oil recovery. They also have potential use in agriculture as seed dressings and soil conditioners. The protective polysaccharide coatings are found to improve germination efficiencies under suboptimal conditions.

A novel environment-friendly seed coating wherein chitosan was modified with sodium hydroxide and polymerized in water with plant growth regulators, microfertilizer and other additives when tested in rice showed an increase in yield and decrease in cost of raw materials consumption [40]. When seeds of Italian ryegrass (*Lolium multiflorum*) and Chinese milk vetch (*Astragalus sinicus*) were coated with a mixture of hygroscopic and plant-derived polysaccharide gums, and inoculated with spores of microorganisms (*Aspergillus sp. and Streptomyces* sp.) serving as coating mixture decomposers, there was an increase in seedling emergence of the coated seeds [41].

Pearl millet seed priming with gum biopolymers, *A. arabica* or *A. indica* exhibitied a growth-promoting effect resulting in increased fresh weight, dry weight, leaf area, plant stand, and grain yield (Figure 16.1) [28]. The other examples of gum biopolymers in plant growth promotion are given in Table 16.2 and Figure 16.2.

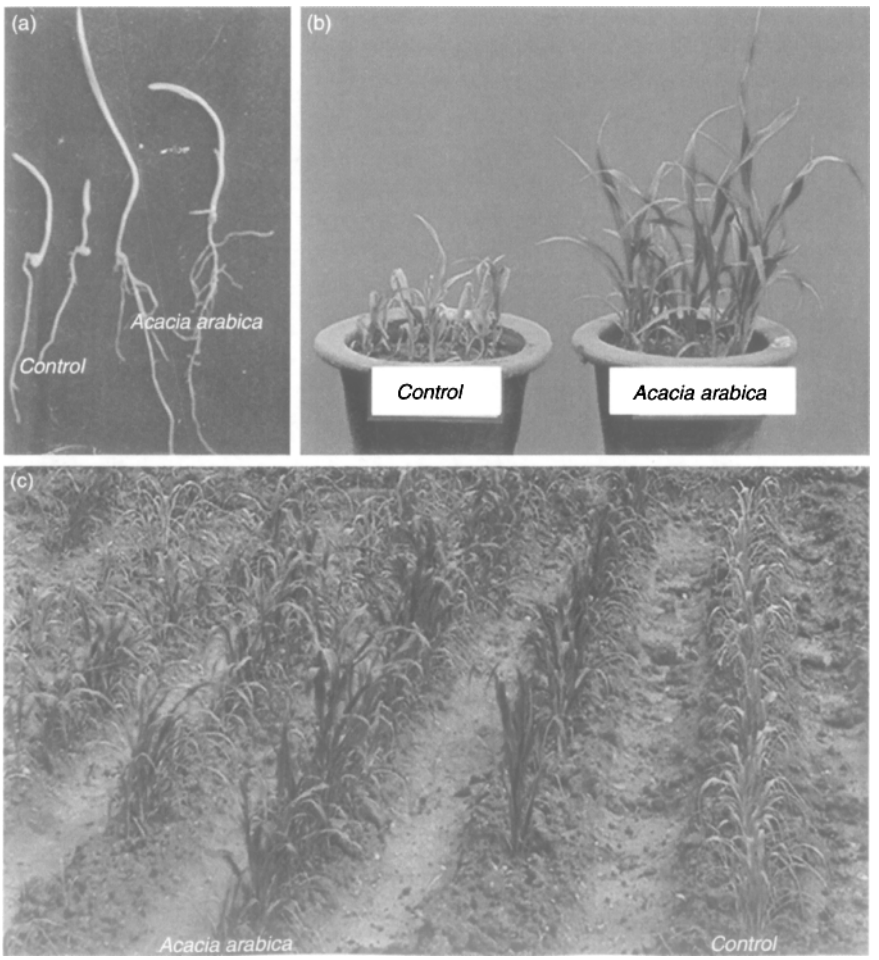

Figure 16.1 Effect of *Acacia arabica* gum treatment on germination and seedling vigor of pearl millet under laboratory conditions (a); Effect of *Acacia arabica* gum treatment on control of downy mildew disease of pearl millet caused by *Sclerospora graminicola* under green house conditions (b) and field conditions (c).

Table 16.2 Efficacy of plant derived gum biopolymers on growth enhancement of various crops.

Crop	Treatment	Results/Remarks	Reference
Pearl Millet	*Acacia arabica*	Enhanced seed germination, seedling vigor, fresh weight, dry weight, leaf area, plant stand and grain yield	[28]
	Azadirachta indica	Enhanced seedling vigor fresh weight, dry weight, leaf area, plant stand and grain yield	[28]
	Moringa oleifera	Enhanced seed germination, seedling vigor, fresh weight, dry weight, leaf area, plant stand	[28]
	Carica papaya	Enhanced seedling vigor	[28]
Sunflower	*Acacia arabica*	Enhanced seed germination, seedling vigor, fresh weight, dry weight, leaf area, plant stand and grain yield	[42]
	Azadirachta indica	Enhanced seed germination, seedling vigor	[42]
	Moringa oleifera	Enhanced seedling vigor	[42]
	Carica papaya	Enhanced seedling vigor	[42]
Tomato	*Acacia arabica*	Enhanced seed germination, seedling vigor, fresh weight, dry weight	[43]
	Azadirachta indica	Enhanced seed germination, seedling vigor	[43]
	Moringa oleifera	Enhanced seed germination, seedling vigor	[43]
	Carica papaya	Enhanced seed germination, seedling vigor	[43]
Chilli	*Acacia arabica*	Enhanced seed germination, seedling vigor, fresh weight, dry weight, leaf area, plant stand and yield	[44]
	Azadirachta indica	Enhanced seed germination, seedling vigor, fresh weight under field conditions	[44]

Table 16.2 (cont.) Efficacy of plant derived gum biopolymers on growth enhancement of various crops.

Crop	Treatment	Results/Remarks	Reference
	Moringa oleifera	Enhanced seed germination, seedling vigor	[44]
	Carica papaya	Enhanced seedling vigor	[44]
Chickpea	*Acacia arabica*	Enhanced seed germination, seedling vigor, fresh weight, dry weight, leaf area, plant stand and yield	[45]
	Azadirachta indica	Enhanced germination, seedling vigor	[45]
	Moringa oleifera	Enhanced seed germination and seedling vigor	[45]
	Carica papaya	Enhanced seedling vigor	[45]

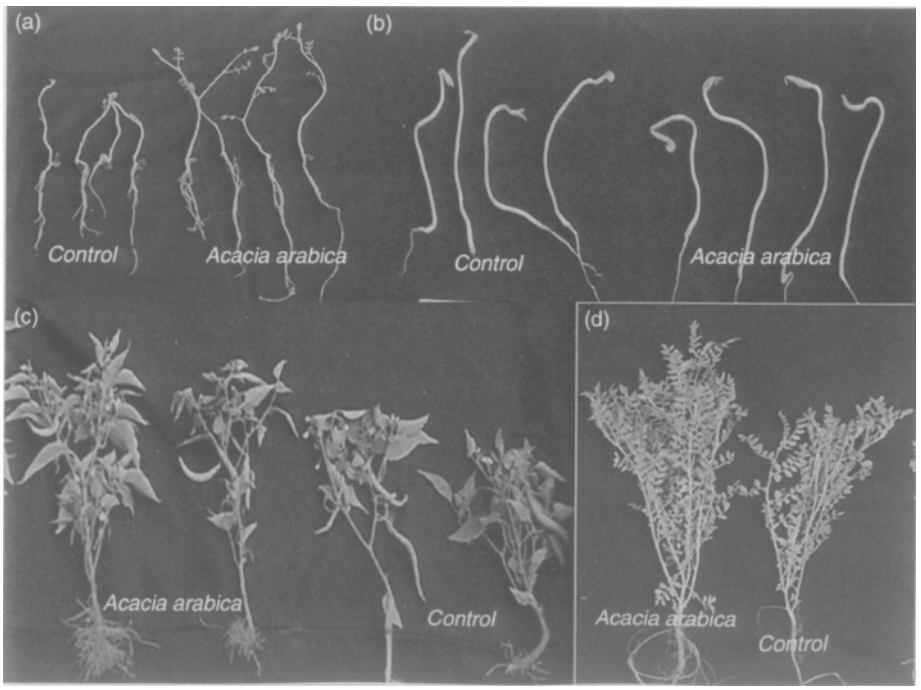

Figure 16.2 Effect of *Acacia arabica* gum treatment on germination and seedling vigor of chick pea (a) and tomato (b) under laboratory conditions; Effect of *Acacia arabica* gum treatment on enhancement of vegetative growth of chilli (c) and chick pea (d) under field conditions.

16.7 Plant Derived Biopolymers in Plant Disease Management

Pearl millet seed priming with gum biopolymers alone provided varied disease protection against downy mildew disease, *A. arabica* or *A. indica* gum along with 3 g kg−1 of metalaxyl 35 SD was the superior treatment, offering significant downy mildew disease reduction (Figure 16.1) [28]. In earlier studies, integration of fungicide metalaxyl XL (at a rate of 0.5 ml kg−1 of seeds) with Striga-mycoherbicides (Foxy2 and PSM197) and resistant maize cultivars using seed treatment technology and gum Arabic adhesive, showed significant reduction in Striga emergence by 81% and 90% compared with the respective resistant and susceptible controls [46].

Specific studies of the use of gum biopolymers directly in plant disease management are very few. Most of the time these gums are used as carriers for biocontrol formulations of *Trichoderma* spp. *Pseudomonas* and *Bacillus* spp. These gums are also used as adhesive for various seed treatment chemicals. A few examples where gum biopolymers were used for plant disease management are given in Table 16.3.

Table 16.3 Efficacy of plant derived gum biopolymers on disease control against different pathogens of various crops.

Crop	Treatment	Pathogen/ Disease	Results/ Remarks	Reference
Pearl Millet	*Acacia arabica*	*Sclerospora graminicola*/ downy mildew	Reduced downy mildew under greenhouse and field conditions	[28]
	Azadirachta indica		Reduced downy mildew under greenhouse and field conditions	[28]
	Moringa oleifera		Reduced downy mildew under greenhouse and field conditions	[28]
	Carica papaya		Reduced downy mildew under greenhouse and field conditions	[28]

Table 16.3 (cont.) Efficacy of plant derived gum biopolymers on disease control against different pathogens of various crops.

Crop	Treatment	Pathogen/ Disease	Results/ Remarks	Reference
Sunflower	*Acacia arabica*	*Plasmopara halstedii*/ downy mildew	Reduced downy mildew under greenhouse and field conditions	[43]
	Azadirachta indica		Reduced downy mildew under greenhouse and field conditions	[43]
	Moringa oleifera		Reduced downy mildew under greenhouse and field conditions	[43]
	Carica papaya		Reduced downy mildew under greenhouse and field conditions	[43]
Maize	*Acacia arabica*	*Fusarium verticilloides*/ Ear rot	Reduced incidence of ear rot under greenhouse and field conditions	[47]
	Azadirachta indica		Reduced incidence of ear rot under greenhouse and field conditions	[47]
Tomato	*Acacia arabica*	*Clavibacter michiganensis*/ bacterial canker	Reduced bacterial canker under greenhouse conditions	[44]

Table 16.3 (cont.) Efficacy of plant derived gum biopolymers on disease control against different pathogens of various crops.

Crop	Treatment	Pathogen/ Disease	Results/ Remarks	Reference
	Azadirachta indica		Reduced bacterial canker under greenhouse conditions	[44]
Chickpea	*Acacia arabica*	*Fusarium oxysporum*/ Wilt	Reduced incidence of wilt disease under field conditions	[44]
	Azadirachta indica		Reduced incidence of wilt disease under field conditions	[44]

16.8 Integrated Use of Plant Gum Biopolymers

A novel corn seed coating agent (NCSCA) was prepared by modifying chitosan, a natural nontoxic biopolymer, trace elements and fertilizer seed treatment which resulted in a high germination percentage and yield in corn, and also showed high resistance to head smut caused by *Sphacelotheca reiliana* stress both under laboratory and field conditions [48].

Plant-based exudates are excellent gum biopolymers which contain plant growth-regulating hormones with priming potential without any side effects. Pearl millet seeds primed with gum exudates of *Acacia arabica, Moringa oleifera, Carica papaya,* and *Azadirachta indica,* were evaluated in combination with metalaxyl (Apron 35 SD) on pearl millet seed quality, growth parameters, and resistance to downy mildew pathogen *Sclerospora graminicola.* Susceptible pearl millet seeds primed with gum biopolymers showed enhanced seed germination and seedling vigor, and *A. arabica and A. indica* gum biopolymers alone or with metalaxyl 35 SD resulted in maximum seed germination [28].

Similarly, plant-based exudates of *Acacia arabica, Moringa oleifera, Carica papaya* and *Azadirachta indica,* were evaluated in biological control bacterial formulations of *Pseudomonas fluorescens* and *Bacillus subtilis* in pearl millet, and it was found that seed quality, growth parameters, and resistance to downy mildew pathogen *Sclerospora graminicola* was observed both under greenhouse and field conditions [28]. It has been found that plant-derived gums used as seed coating polymers can provide excellent water-soluble or water-dispersible film, are

stable in storage, and have good adherence to seed. A number of trees like *Acacia arabica, Moringa oleifera, Carica papaya,* and *Azadirachta indica* are well known to produce gum exudates during their different stages of growth. An approach employing these benefits can minimize the recommended dose of fungicides by reducing dust-off, and provide excellent control on plant pathogens [49]. The gum biopolymers have particular application in the protection of seeds against fungal diseases when combined with one or more fungicide [50]. Biopolymers dry quickly, dissolve rapidly in water, and do not inhibit germination.

Application of some natural polymers as seed treatment with a reduced dose of recommended metalaxyl 35 SD fungicide would prove beneficial, cost effective, eco-friendly and could be a potential component of integrated pest management. These natural polymer seed coatings, in addition to their action against downy mildew, are interesting in that they can be good growth promoters, which is an added advantage. Such products and formulations can be safer and more convenient to use, more effective at much lower rates, less toxic to non-target species, and have a lower impact on the environment generally [51]. These compounds may provide the user with a convenient, safe product that will not deteriorate over a period of time, along with the means to obtain the maximum activity inherent in the active ingredient.

16.9 Transgenically Produced Biopolymers

The genetic manipulation of microorganisms opens up an enormous potential for the biotechnological production of biopolymers with tailored properties suitable for high-value medical applications such as tissue engineering and drug delivery [52]. Plants are becoming factories for the production of biopolymers. Researchers created a *Arabidopis thaliana* plant through genetic engineering. The plant contains the enzymes used by bacteria to create biopolymers. Bacteria create the plastic through the conversion of sunlight into energy. The researchers have transferred the gene that codes for this enzyme into the plant; as a result the plant produces plastic through its cellular processes. The plant is harvested and the biopolymers are extracted from it using a solvent. The liquid resulting from this process is distilled to separate the solvent from the biopolymer. Increasing environmental concerns have fuelled research into novel renewable and environmentally benign polymers, including their production in transgenic plants [53]. Transgenic plants can assemble and accumulate many valuable proteins that can be economically extracted or processed. They are showing considerable potential for the economic production of biopolymers, with a few already being marketed. Our increasing understanding of protein targeting and accumulation should further improve the potential of this new technology [54]. Apart from economic advantages, there are qualitative benefits favouring the use of transgenic plants as factories for producing recombinant proteins, in particular for biopolymers [55, 56, 57]. One such biodegradable protein- based polymer has been recently produced in tobacco [58, 59]. In order to genetically modify microorganisms

capable of producing polyhydroxyalkanoate (PHA) biopolymers, a simple and rapid method to prepare freshly plated *Pseudomonas* cells for transformation via electroporation was developed. Furthermore, this transformation procedure can be performed in less than 10 min, saving a great deal of time compared with traditional methods [60]. Genetic engineering of plants for the production of novel polymers and platform chemicals can help to alleviate the demands for limited resources and potentially provide a platform to produce valuable compounds in bulk quantities. However, the success of transgenic plants as bioreactors depends on competitive high-yield production capacities. Recent advances in enhancing the production of novel compounds in transgenic plants include multigene transformation and the direction of biosynthetic pathways to specific intracellular compartments. It now appears feasible to produce interesting proteins such biopolymers that could replace petroleum-based plastics using transgenic plants. Direct production of novel compounds in biomass crops with the aim to produce bioenergy as a coproduct provides a promising way to improve the economics of transgenic plants as biopolymer biofactories [61].

16.10 Conclusions and Future Prospects

Naturally derived biopolymers are biocompatible, biodegradable, and versatile and have found applicability in all fields of agriculture while becoming vital in terms of environmental and human safety. Novel families of biopolymers can be produced in significant quantities from lower and higher plants, microbes and animals, and also the readily available renewable agricultural wastes. Advances in the field of biotechnology and related sciences such as genetic engineering and recombinant DNA technology have changed the ways of synthesis of biopolymers. Modern biotechnology tools like recombinant DNA techniques permit the creation of biopolymer chains that are virtually uniform in length, composition, and stereochemistry, and allow creation of new materials more economically. Biotechnology enables modifying genes, and it is now possible to genetically modify an organism so that it produces greater quantities of a particular biopolymer. At present, several new biopolymers are being developed for applications in the field of agriculture, particularly for seed coating, precision sowing, and value addition to seeds. Higher value seeds are subjected to biopolymer based fungicide coating which offers broad-spectrum protection against major soil-borne and seed-borne pathogens. More emphasis will be placed on the exploration of biopolymers for plant disease management programs and incorporation of biopolymer technology into integrated pest management strategies. Biopolymer applications in agriculture are increasing and new beneficial uses have been found. However, more research is needed to strengthen expertise in biopolymer engineering and genetic modification of biological system to produce specifically tailored biopolymers for high-tech agriculture. Biopolymer production has tremendously advanced and biopolymers are being designed for catering to all kinds of specific applications. However, in spite of their environmentally safe appeal, their production

costs have to be brought down by strengthening research and development efforts in the biopolymer area.

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17

Modified Cellulose Fibres as a Biosorbent for the Organic Pollutants

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Abstract

This chapter summarizes the most recent advances in the realm of the use of lignocellulosic material, either in its natural form or after chemical modification of the surface, as biosorbent for the uptake of dissolved organic pollutants. The adsorption capacity of several natural lignocellulosic products such as agricultural by-products and waste materials for aromatic organic pollutants including pesticides will be explored in the first part of the chapter. Then, the emphasis will be placed on the different strategies of target modification of the fibres' surface in view of the enhancement of the adsorption capacity and selectivity toward a wide range of organic pollutants. Many of the modified cellulose adsorbents proved regenerable and re-usable over a number of adsorption/desorption cycles allowing recovery of the adsorbed organic pollutant in a more concentrated form. The driving forces related to the use of cellulose fibres in these fields of applications reside in their biorenewable character, their ubiquitous availability in a variety of forms, and their low cost.

Keywords: Lignocellulosic sorbent, adsorption, organic pollutant, chemical modification of cellulose, biosorbent

17.1 Introduction

Adsorption is one of the most frequently applied methods to remove pesticides and dissolved organic pollutants and other hazardous chemicals from water, due to its efficiency, capacity, and possible applicability on a large scale.

Adsorption is a well-known equilibrium separation process. It is now recognized as an efficient and economic method for water decontamination applications and for separation analytical purposes. Generally, a suitable substrate for adsorption processes of pollutants should meet several requirements: (i) High adsorption efficiency; (ii) adsorption capability for the removal of a wide variety of organic compounds; (iii) high capacity and rate of adsorption; (iv) important selectivity for different concentrations; (v) granular or powder form with high surface area; (vi) good mechanical and structural integrity

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (483–524)

(vii) able to be regenerated without loss of the adsorption capacity; (viii) tolerance for a wide range of wastewater parameters; (ix) and low cost.

Activated carbon is the most commonly used adsorbent for the removal of toxic organic substances from water. However, in spite of its prolific use, activated carbon remains an expensive material whose capacity fluctuates according to the raw materials used for its production, and the higher the quality of activated carbon, the higher is its cost. Today, there is a growing interest in developing natural low-cost alternatives to synthetic polymers. Lignocellulosic-based materials can be used for this purpose. Their biorenewable character, their ubiquitous availability in a variety of forms, and their low cost represent a lot of potential inciting their use in the field of water treatment. In particular, the increasing cost of conventional adsorbents undoubtedly makes biopolymer-based materials one of the most attractive biosorbents for wastewater treatment. A number of cheaper materials, including industrial and agricultural wastes, have been used to remove different pollutants from industrial effluents for their safe disposal into the biosphere. In addition to their ability to uptake toxic metals, lignocellulosic-based material demonstrated the capacity to remove organic pollutants. However, despite the large number of papers dedicated to the application of biopolymers in the field of adsorption, most of them focus on the adsorption of toxic metals and only a few were concerned with dissolved organic pollutants such as pesticides, herbicides, organic solvents, and the like.

In the first part of this chapter, a survey of the most relevant works regarding the use of different natural lignocellulosic products as a biosorbent for various organic pollutants is presented. The adsorption capacity, the pollutant model used, and their regeneration aptitude are reported. Then a detailed investigation of the different strategies of chemical modification carried in view of the enhancement of the adsorption capacity of cellulosic fibres, with the possible regeneration and reuse of the sorbent is fully described.

17.2 Cellulose Structure

Cellulose is not only the most available natural polymer on earth, but also one of the most interesting, naturally existing, supramolecular structures. The particular 3D network formed by hydrogen bonds leads to a complex structure formed by nanodomains of crystalline structure co-existing with amorphous parts. This crystalline structure is responsible for its intrinsic strength and its relatively high chemical stability. The ensuing ultrastructure of cellulose fibres is also responsible for the adsorption properties of lignocellulosic material. Information on this assembly mode in nanoscale (level) is essential for a deeper understanding of the unique properties of wood.

17.2.1 Molecular Level

The cellulose chemical formula is $C_6H_{10}O_5$. The widely accepted macromolecular structure of cellulose is given in Figure 17.1. The glucose base units are

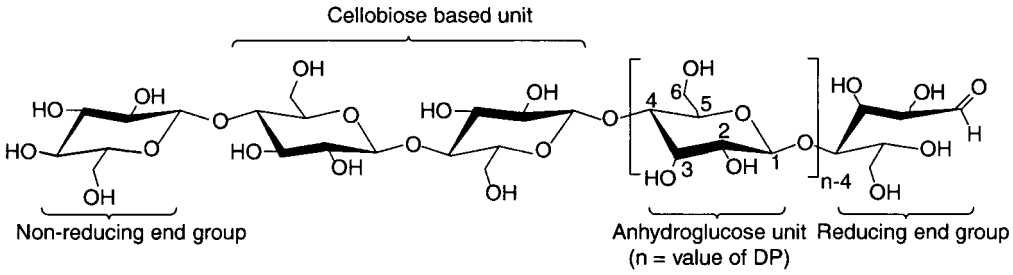


Figure 17.1 Molecular structure of cellulose.

linked together by β-1,4 glycosidic bonds formed between the carbon atoms C-1 and C-4 of adjacent glucose units.

Cellulose is a linear syndiotactic homopolymer composed of D–anhydroglucose units (AGU), which are linked together by β–1,4–glucosidic bonds formed between the carbon atoms C(1) and C(4) of adjacent glucose units. The β–link requires that the plane of the pyranose ring of each second glucose unit along the molecular chain is turned around C(1) – C(4) axis by 180° with respect to the glucose units lying in between.

The chain length of cellulose expressed in the number of AGU constituents (degree of polymerization, DP or n) varies with the origin and treatment of the raw material. In case of wood pulp, the values are typically between 300–1700. Cotton and other plant fibres have DP values in the 800–10000 range, depending on treatment.

The molecular structure imparts cellulose with its characteristic properties such as hydrophilicity, chirality, degradability, and broad chemical variability initiated by the high donor reactivity of the OH groups. It is also the basis of the extensive hydrogen bond networks, which give cellulose a multitude of partially crystalline fibre structures and morphologies.

Native cellulose has DP varying upon the cellulose source. It can be higher than 10,000 for cotton. Depending on the severity of cooking and pretreatment, cellulose used in dissolving wood pulps has an average DP of 600–1200 and 100–200 for cellulose powders. The three hydroxyl groups of the AGU are able to interact and form hydrogen bonds by means of intra- and intermolecular interactions. The strength of these hydrogen bonds is around 25 kJ/mol [1].

Intramolecular hydrogen bonding is the main cause of the relative stiffness and rigidity of the cellulose molecule, which is reflected in its high tendency to crystallize and its ability to form fibrillate strands. The chain stiffness is further favored by the β–glucosidic linkage, which, in contrast to the α–glucosidic bond in starch, predetermines the linear nature of the chain, and by the chair conformation of the pyranose ring.

17.2.2 Supermolecular Structure

As described before, the cellulose chains have a strong tendency to aggregate to highly ordered structural entities due to their chemical constitution and

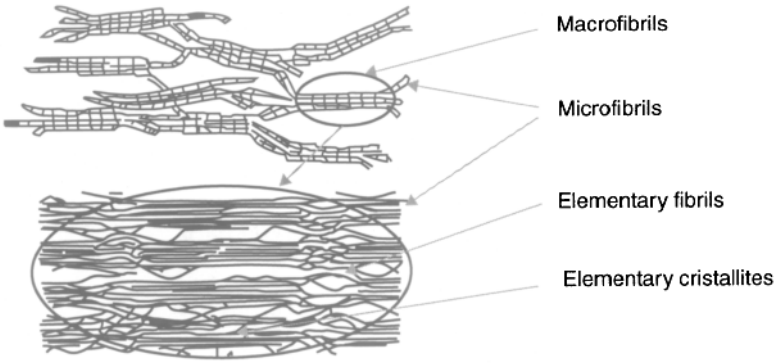


Figure 17.2 Representation of the fringed fibrillar model for the fibre structure[3].

their spatial conformation. The basic elements of the supermolecular structure of cellulose fibres are the crystallite strands. The cellulose chains are grouped in microfibrils which are parallel, giving a more densely packed arrangement, and are aligned more or less with the fibre axis.

Among the different descriptions of the structure model for the internal build-up of cellulosic fibres, the one given by Hearle [2] is the most illustrative. This model assumes low ordered (amorphous) and highly ordered (crystalline) regions arranged in a fringed fibrillar fashion. As shown in Figure 17.2, according to this model, the fibrous substrates are built from fibrillar elements, which are known as 'macrofibrils' and 'microfibrils'. The microfibrils with 10–20 nm in width are an aggregation of elementary fibrils called elementary crystallites with a typical lateral dimension of 3–7 nm and length of 100–300 nm depending on the fibres origin. The elementary fibril is formed by the association of many cellulose molecules (between 20–60 chains), which are linked together in repeating lengths along their chains. This particular structure gives rise to a large void or internal microporosity of nanometer dimensions between the fibrils as a result of the imperfect axial orientation of the fibrillar aggregates, and by the less ordered interlinking regions between the crystallites inside the elementary fibrils. The supramolecular structure is described in terms of crystalline and non-crystalline regions by the degree of crystallinity, orientation, and dimensions of these regions [3].

17.2.3 Ultrastructure

The cellulose fibre is organized in a cellular hierarchical structure and can be described as a multiphase system of concentric layers surrounding the lumen. The outermost layer is the primary wall (P), followed by the outer layer of the secondary wall (S1), the middle layer of the secondary wall (S2), and the inner layer of the secondary wall (S3), as shown in Figure 17.3. The middle lamella (ML) which is located outside the primary wall is not considered a cell wall layer.

The cellulose microfibrils are aligned fairly parallel in a helical pattern within each layer in the cell wall and oriented at different microfibril angles in the

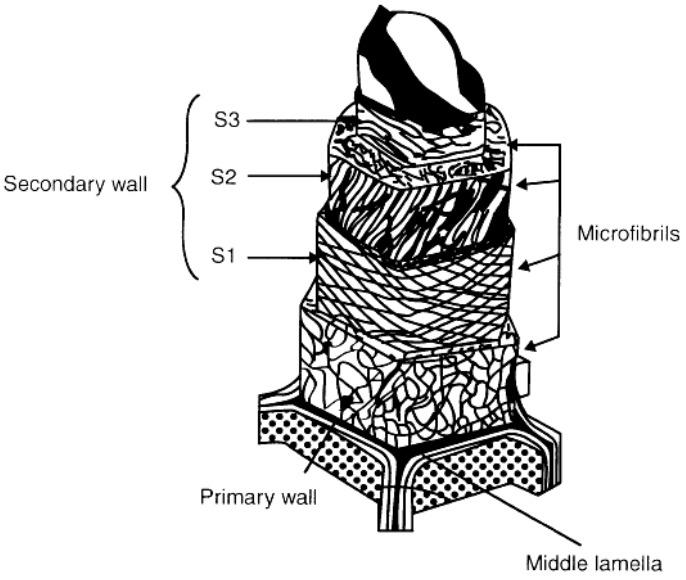


Figure 17.3 Typical morphology of Cellulose fiber.

different layers, where the microfibril angle (MFA) is the angular deviation of the microfibrils from the longitudinal fiber axis. The middle lamella (0.5–2 μm) is mainly composed of lignin (70%), associated with small amounts of hemicelluloses, pectins, and cellulose. The primary wall, often hard to distinguish from the middle lamella, is very thin (30–100 nm) and is composed of lignins (50%), pectins, and hemicelluloses. The secondary wall is the main part of the vegetal fibres. Its essential component is cellulose and it bears three layers, namely, the external, S1 (100–200 nm), the central S2 (the thickest layer of 0.5–8 μm), and the internal or tertiary layer, S3 (70–100 nm) situated close to the lumen.

Cellulose is distinguished by the multiple hydroxyl groups (six per unit), which accounts for its hydrophilic properties. These OH groups are responsible for the hydrogen bonds network holding firmly the chains together side-by-side and forming microfibrils with high tensile strength. The tight array of inter- and intra-hydrogen bonds between the cellulose chains along with the high crystalline structure is responsible for the strength and cohesion of the cellulose fibres making them insoluble in most of the conventional solvent cellulose, and hard to break down into free individual cellulose chains as strength imparts rigidity, where the microfibrils are meshed into a carbohydrate matrix, allowing for rigidity to plant cells.

In addition to the fibrillar morphology of the fibre cell wall, the fibres are characterized by capillaries, voids, and interstices providing the cellulose fibres a highly porous character. The pore size ranged from 5 up to 30 nm and the pore volume fraction attained 1–3% for cotton and wood pulp. However, the total pore volume and pore size distribution are very sensitive to pretreatments. Mercerization leads to a decrease in pore diameter and an enhancement of micropore surface, while enzyme treatments enlarge the existing pores [4].

Acid hydrolysis enhances the pore system by removing amorphous cellulose from the surface and revealing the macrofibrillar structure of cellulose fibres [5]. Drying results in an irreversible reduction of the pore volume as a result of the pores collapse arising from the capillary forces, a mechanism called hornification.

Due to the pore and void system, the surface area of cellulose fibres exceeds by far the geometrical outer surface of the fibre. The inner surface area is considered a key parameter with regard to accessibility and adsorption behaviour of lignocellulosic material in their native form as well as in a modified form.

The abundance of OH functionalities brings to cellulose a high reactivity and opens the way to a multitude of modification chemical reactions calling on the OH chemistry. Moreover, cellulose has the particularity of being a porous material, the extent of which is boosted after water expansion, and this property plays an important role in the adsorption process.

Cellulose was used for a long time as a native sorbent to remove heavy metal ions. Yet rarely was it used to uptake the organic pollutants [6, 7, 8].

17.3 Application of Natural Lignocellulosic Materials as Adsorbents for Organic Pollutants

A large number of natural lignocellulosic materials have been used as adsorbents for the uptake of some organic pollutants under various circumstances. The adsorption process seems to be related to many factors such as the chemical structure of the substrate, its hydrophobicity, its porosity and surface area, the structure of the pollutant, its water solubility and its geometry. Xing *et al.* [8] have investigated the relation of α-naphthol uptake with the polarity and aromaticity of selected organic matter surrogates using batch equilibration. Among the studied sorbents, cellulose and lignin have been tested and they have shown from linear isotherms that partition of α-naphthol to cellulose and lignin reached equilibrium from 48 h to 2 weeks, respectively. The slow partition rate observed in the case of lignin may be attributed to the low wettability of this biopolymer. The maximum adsorbed amount was about 5 $\mu mol.g^{-1}$ for cellulose and 75 $\mu mol.g^{-1}$ for lignin. The relatively low adsorption onto cellulose has been associated with the strong polar interactions of cellulose with water, where wetting energy is about 20 $J.g^{-1}$, and with its low aromaticity compared to lignin. Another study has been carried out by Barrera-Garcia *et al.* [9] who investigated the sorption and diffusion properties of phenolic compounds diluted in hydro-alcoholic solution by individual wood macromolecules; cellulose, hemicellulose and lignin. Kinetic experiments have shown that the time to reach the sorption equilibrium in the presence of wood polysaccharides (cellulose and hemicellulose) varied from some hours up to 5–7 days in the presence of lignin. This difference in the equilibration times is probably due to a faster diffusion towards the polysaccharide sorption sites. Indeed, the sorption process has been controlled by Fick's law of diffusion. From all the tested phenolics, the highest sorption level is for lignin (up to 60%), then for hemicellulose (40%), and the lowest is for cellulose (30%). This investigation highlighted

the huge dependency of the adsorption process on the chemical structure of the sorbate.

A number of cheaper materials, including industrial and agricultural wastes, have been used as low cost adsorbents to remove different pollutants from industrial effluents for their safe disposal into the biosphere. In this context, Achaka *et al.* [10] showed that the banana peel displayed a high adsorption capacity of phenolic compounds (about 690 $mg.g^{-1}$), revealing that banana peel could be employed as a promising adsorbent for phenolic compounds adsorption. The adsorption process was relatively fast, reaching equilibrium within 3 h contact time. The adsorption capacity reached about 80 $mg.g^{-1}$ in the presence of 3 $g.100\ mL^{-1}$ of banana peel. The authors also carried out the adsorption dependency of pH, and they found that pH played an obvious role on the phenolic compounds adsorption capacity onto banana peel. An increase of the solutions' pH leads to a significant rise in the adsorption capacities of phenolic compounds on the banana peel. Both Langmuir and Freundlich isotherms have been tested, and they provide good correlations for the adsorption of phenolic compounds onto banana peel. Kinetics showed that the pseudo-second-order kinetic model represented the experimental data better than the pseudo-first-order model and intraparticle diffusion with a better fit. Desorption experiments showed an almost chemisorption interaction between the natural phenolic and the adsorption sites on the banana peel.

Akhtar *et al.* [11] carried out a large investigation on the use of diverse natural biosorbents for the uptake of different model organic pollutants. Rice husk has been used as a low cost sorbent for the removal of 2,4-dichlorophenol. The maximum adsorbed amount reached about $7\mu mol.g^{-1}$ and the equilibrium adsorption kinetic occurred within 10 min. Freundlich and Langmuir's isotherms revealed better sorption at low concentrations, and it was shown that the sorption process had a physisorption nature. They also carried out a thermodynamic study which showed the exothermic and spontaneous nature of the sorption process on the surface of rice husk. Methanol was found to be a more suitable solvent for desorption of the sorbate. An application of the 2,4-dichlorophenol removal from industrial wastewater collected directly from the outlet of a pharmaceutical and paper mill using rice husk as sorbent has been carried out. The sorption potential of Moringa Oleifera pods to remove different aromatic organic compounds including cumene, ethylbenzene, toluene, and benzene from aqueous media has also been tested [12]. The data have been fitted with different adsorption models. The adsorption capacity of the substrate ranged from 50–100 $mmol.g^{-1}$ with an equilibrium adsorption kinetic being lower than 1h. The organic pollutants sorption depended directly on their solubility. It has been shown from the sorption isotherm obtained from Dubinin-Radushkevich (D-R) model that the sorption process was governed mainly by Van der Waals interaction. Thermodynamic study carried out demonstrated the stability of the sorption process which was exothermic and spontaneous. The results of the Lagergren plot followed first-order rate equation. The trapped solutes could be desorbed by washing with methanol.

Adachi *et al.* [13] suggested that the use of beer bran as an adsorbent is an efficient and cost-effective method to remove several organic compounds such

as dichloromethane, chloroform, trichloroethylene, benzene, and pesticides from wastewater. The retention ratios ranged from 75 up to 95%, and the adsorption efficiency of beer bran for benzene, chloroform, and dichloromethane was higher than that of activated carbon.

Another study carried out by Memon *et al.* [14] was interested in the removal of a pesticide using a biopolymer. About 24 $\mu mol.g^{-1}$ of methyl parathion pesticide were adsorbed by watermelon peel through mainly a physicosorption process. The kinetics of adsorption follows a first-order rate equation. Thermodynamic parameters ΔS, ΔG, and ΔH indicated that adsorption process is thermodynamically favourable, spontaneous, and endothermic in nature. Sorptive potential of selected agricultural waste materials, i.e. rice (Oryza sativa), bran, bagasse fly ash of sugarcane (Saccharum officinarum), Moringa oleifera pods, and rice husk for the removal of methyl parathion pesticide has also been investigated [15]. A relatively low adsorption capacity being in the range of 0.5–5 $mmol.g^{-1}$ according to the tested sorbent has been observed. Significant increase in the sorption capacity by 10 up to 45% has been noted after the chemical treatment of the substrate with nitric acid and methanol which was explained in terms of the expansion of the pore volume and surface area of the sorbents. The adsorbed pesticide may be stripped off by sonication with methanol, making the regeneration and reuse of sorbents possible.

In a study achieved by Memon *et al.* [16] the sorption of carbofuran and methyl parathion on treated and untreated chestnut shells has been studied using high performance liquid chromatography. In this study, the maximum sorption of methyl parathion and carbofuran onto chestnut shells was achieved at a concentration of $0.38.10^{-4}$ and $0.45.10^{-4}$ $mol.dm^{-3}$ respectively. Adsorption isotherms depicted a better fitting with the Langmuir isotherm. The results of sorption energy obtained from the Dubinin-Radushkevich isotherm pointed out that adsorption was driven by physical interactions. The kinetics of sorption follows a first-order rate equation. The thermodynamic parameters ΔS and ΔG indicate that the sorption process is thermodynamically favourable, and spontaneous, whereas the value of ΔH shows the exothermic nature of sorption process for methyl parathion and endothermic nature of carbofuran. The developed sorption method has been employed in methyl parathion and carbofuran in real surface and ground water samples. The sorbed amount of methyl parathion and carbofuran may be removed by methanol to the extent of 97–99% from the surface of chestnut shells.

Le Bourvelleca *et al.* [17] have investigated the sorption of procyanidins, a polyphenol tannin derivative, on native cellulose fibers and other polysaccharide substrates such as modified pectin and xyloglucan and starch. Through this study, they showed a relatively low affinity of the substrate towards the tested chemical which presented a low adsorbed around 4 $mg.g^{-1}$.

The sorption of aromatic compounds on cyclodextrin–EPI copolymer has been developed by Yu *et al.* [18]. They found that this copolymer has high extraction efficiency with recoveries between 90 and 100% for aromatic compounds. Other highly crosslinked cyclodextrin-EPI copolymer has been tested for the adsorption of bisphenol A and more than 98% was removed [19].

Sorption of phenanthrene and naphthalene by chitin powder and cellulose fibers, as well as the ensuing chars obtained by partial heat combustion, was examined [20]. Compared to native cellulose and chitin sorbent for which the adsorption capacity was attained, the charring treatment greatly enhanced the adsorption capacity which climbed from 0.15 up to 160 $mg.g^{-1}$ for naphthalene and from 0.15 up to 60 $mg.g^{-1}$ for phenanthrene. The adsorption process was explained by the accumulation of the adsorbed molecules within the inner substrate micropores. However, the surface properties, namely the hydrophobic characteristic, also govern the adsorption process accounting for the more efficient adsorption behaviour of the charred materials. Nonetheless, the reuse aptitude of the sorbent once exhausted has not been highlighted. Table 17.1 summarises the principle results presented above.

17.4 The Use of Modified Cellulose Fibres as a Sorbent for the Organic Pollutants Removal

The use of cellulose as adsorption support is not recent. Previous works highlighted the ability of this natural material to adsorb a certain number of organic compounds, such as pesticides [21, 22] and organic dyes [22, 23]. The ability of cellulose fibres to adsorb metal ions has also been well reported [24, 25]. However, the adsorption properties of native cellulose for organic pollutants and metallic contaminants remain very low (from 100–500 times lower) in comparison to conventional sorbent such as activated carbon, zeolite, or organophile clays. This effect is mainly associated on one hand to the low concentration of active sites on which organic pollutant could be adsorbed, and on the other hand to a lower accessible porosity of the lignocellulosic material. In order to enhance the adsorption capacity and effectiveness, cellulose ought to undergo a surface modification with the aim of generating new adsorption sites on which organic pollutant molecules could be accumulated or adsorbed through Van der Waals interaction. Conversely, physicochemical treatment aiming to favour the accessibility of the internal pore to pollutant diffusion is also an alternative to enhance the adsorption capacity of the substrate.

17.4.1 Adsorption of Model Organic Compounds on Surfactant Treated Cellulose Fibres

Among the original strategy aiming to use deliginified cellulose fibres as a support to trap organic pollutants dissolved in water, one can cite the work carried out by Boufi *et al.* who called on the generation of admicelles around cellulose fibres using cationic surfactants [27, 28, 29] or grafted surfaces [30, 31, 32, 33]. The purpose of this strategy was to exploit these admicellar sleeves as hydrophobic reservoirs for water-insoluble organic molecules. The first study of this series [27–29] showed that bleached cellulose fibres treated with octadecyltrimethylammonium bromide (ODTMA) boosted by a factor 4 the adsorption capacity of 2-naphthol compared to untreated fibres. As shown in

Table 17.1 Survey of the most relevant natural lignocellulosic based sorbent used for the adsorption of different organic pollutant.

Ref.	Authors	Natural lignocellulosic sorbent	Organic pollutant	Results		
				Adsorption equilibrium time	Q_{max} (maximum adsorbed amount)	Other considerations
8	Baoshan Xing *et al.* (1994)	Cellulose	α-naphthol	48 h	5 μmol.g^{-1}	Dependency of the adsorption process on the polarity and aromaticity of the sorbent
		Lignin		2 weeks(low wettability)	75 μmol.g^{-1}	
9	Garcia D.B. *et al.* (2008)	Cellulose Hemicellulose	Phenolic compounds	Some hours	30% 40%	Huge dependency of the adsorption process on the chemical structure of the sorbate
		Lignin		5-7 days	Up to 60%	
11	Achaka *et al.* (2009)	Banana peel	Phenolic compounds	3 h	690 mg.g^{-1}	Pseudo-second-order kinetic model, chemiosorption process
12	Mubeena A. *et al.* (2006)	Rice husk	2,4-dichlorophenol	10 min	7μmol.g^{-1} (99%)	Physiosorption process, pseudo-first kinetic model, exothermic and spontaneous process
13	Mubeena A. *et al.* (2007)	Moringa Oleifera pods	Organic aromatic compounds	Lower than 1 h	50–100 mmol.g^{-1}	ion-exchange mechanism, pseudo-first kinetic model, exothermic and spontaneous process, desorption by methanol wash

14	Adachi *et al.* (2006)	Beer bran	Organic compounds	90 min	76–95%	Freundlich type of adsorption isotherms, adsorption capacity higher than that of activated carbon
15, 16	Memon G.Z. *et al.* (2008)	Watermelon peel	Methyl parathion pesticide	60 min	24 $\mu mol.g^{-1}$	physic-sorption process, pseudo-first kinetic model spontaneous and endothermic process, possible regeneration and reuse of sorbents by methanol wash
		Agricultural waste materials				
17	Memon G.Z. *et al.* (2007)	Chestnut shells	Carbofuran	30 min	$4.5.10^{-5}$ $mol.l^{-1}$	Physisorption process, pseudo-first kinetic model spontaneous and endothermic process for carbofuran and exothermic for methyl parathion
			methyl parathion		$3.8.10^{-5}$ $mol.l^{-1}$	
18	Bourvelleca *et al.* (2005)	Cellulose	Procyanidin	60 min	400 $mg.g^{-1}$	Structure and conformational organisation could play an important role in adsorption process
22	Wang X. and Xing B. (2007)	Cellulose and chars	naphthalene	5–9 days	0.15 to 160 $mg.g^{-1}$	the charring treatment greatly enhanced the adsorption capacity

Figure 17.4, in the absence of adsorbed ODTMA surfactant, the adsorption of 2-Naphthol does not exceed 20 μmol.g^{-1} on the cellulose fibres. On the other hand, retention of 2-naphthol increased with the amount of ODTMA adsorbed up to a certain surfactant concentration close to the critical micellar concentration (CMC), then levelled off rapidly to a very small value. This behaviour has been correlated with the surfactant aggregation at the cellulose/water interface [26, 27, 29].

The large enhancement of solute uptake was the result of an adsolubilization process within the surfactant/cellulose aggregative domains generated by the adsorption of the surfactant molecules giving rise to admicelle or hemimicelle. The adsolubilization process can be viewed as the uptake of dissolved organic compounds into adsorbed surfactant aggregates acting as hydrophobic cores formed by the associated surfactant tails on which organic compounds can be trapped. This phenomenon is comparable in its description to the solubilization of organic compounds into micelles generated by the surfactant in aqueous solution. Hemimicelles have been viewed as a local surfactant monolayer oriented with their head groups in contact with the aqueous media, whereas admicelles are associated with the formation of a surfactant bilayer bearing the lower layer head groups adsorbed on the surface and the head groups of the top layer oriented toward the solution. The decrease of 2-naphthol coadsorption beyond a critical surfactant concentration close to CMC suggests that once free micelles were formed the solute was preferentially incorporated by these microreservoirs, rather than being adsorbed by surfactant present on the fibres. The kinetics of 2-naphthol coadsorption was achieved within 30 min.

The adsorption potential of cationic surfactant-treated cellulose fibres was extended to a large series of organic pollutants differing by their chemical structure.

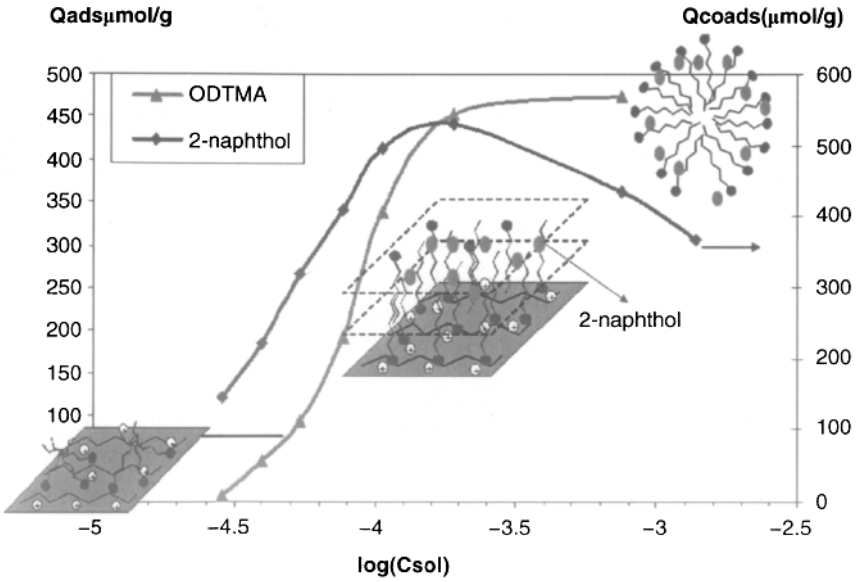


Figure 17.4 Adsorption isotherm of ODTMA and coadsorption of 2-naphthol onto cellulose fibers[24].

In all instances, similar trends have been observed for all the components irrespective of their structure (Figure 17.5). Noticeably enhanced solute uptake was noted up to a critical ODTMA surfactant concentration of about 0.25 mmol.L^{-1}, which seemed to be independent of the organic solute. Above the CMC of the surfactant, a continuous decrease of the amount coadsorbed is equally apparent for all the solutes. The maximum amount of solute adsolubilized varied from 170 to 300 µmol.g^{-1}, depending on the solute's chemical structure. Adsolubilization of solutes was a consequence of their accumulation on the hydrophobic core of adsorbed surfactant aggregates, and not an issue of a specific interaction between the head surfactant groups and the polar moiety of solute.

In order to further enhance the adsorption capacity of the surfactant-treated fibres toward organic pollutants, the density of carboxylate groups along the fibres was amplified through TEMPO-mediated oxidations [28]. Considering that carboxylic groups on the surface is the precursor toward the aggregation of the surfactant molecules, the increase in the surface concentration of the anionic site will bring about further growth of aggregative domains formed by adsorbed surfactant molecules on which organic compound could be trapped. From these investigations [31] it was shown that:

- The adsorption capacity of the surfactant treated fibres is enhanced by the oxidation action of the cellulosic fibres. Thus, for example, the maximum uptake of 2-naphthol grows from 175 to 1040 and 1150 µmol.g^{-1} of cellulose fibres bearing 0, 150 and

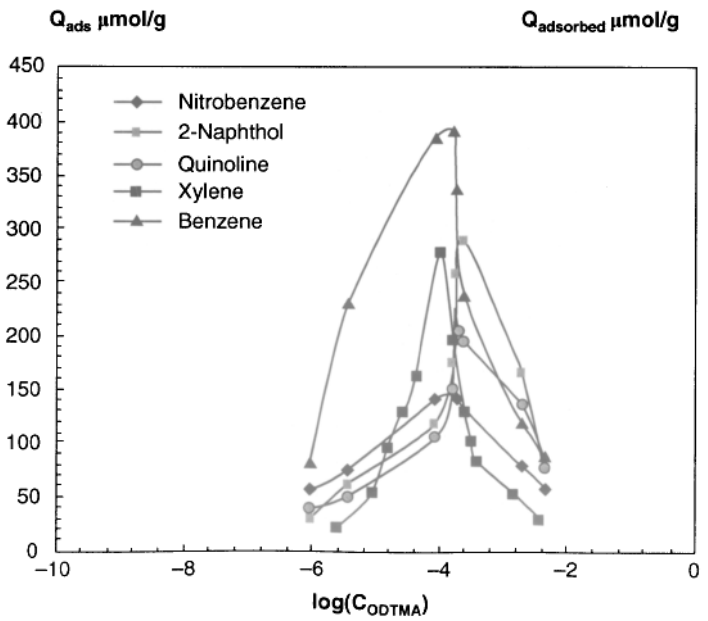


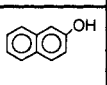
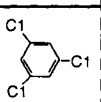
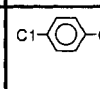
Figure 17.5 Coadsorption isotherms of different organic solutes onto cellulose fibers after treatment with ODTMA, at pH 6.5–7[24].

650 μmol.g^{-1} of COOH groups respectively, and in the presence of hexadecyltrimethylammonium bromide as a cationic surfactant. The same trend was noted for other organic compounds as shown in Figure 17.6.

- Different water-insoluble organic pollutants can be successfully sucked into the aggregative domains by the adsolubilization process as shown in Table 17.2.
- The longer the hydrophobic tail of the surfactant, the better the adsolubilization capacity of the modified surface. Thus, for example, the maximum adsolubilized quantities of 2-naphthol were 290, 180, 140, and 120 μmol.g^{-1} for cationic surfactant bearing 18, 16, 14, and 12 methylene groups, respectively (Table 17.2, Figure 17.6).

Surfactant-modified cellulose fibres appeared to be effective sorbents for the removal of organic compounds dissolved in aqueous media. Given the ready availability of this type of substrate and its low cost, the present approach could be promising in applications related to the removal of organic pollutants and toxic substances in wastewaters. However, the adsorbed surfactant molecules were found to desorb from the cellulose surface during the recycling of the substrate [29], limiting its possible use for multiple adsorption cycles in a

Table 17.2 Maximum amount (μmol.g^{-1}) of adsorbed organic molecules onto surfactant treated cellulose fibers[25].

adsorbent	surfactant	2-N	TCB	NTR	QNL	CBZ	DCB	Ref.
Cel-0	C16	180	147	125	150			29
	C18	290	185	194	202	310		30
Cel-150*	C16	530	540	470	550	120	250	29
	C12	480	460	445	–			29
Cel-450	C16	890	850	570	650	300	380	29
Cel-600	C16	980	880	680	–	436	460	29
	C12	898	797	665	–	400	390	29
Water solubility (mmol/l)		5	16.9	0.22	46	4.46	0.4	
Chemical Structure								
Ln(K_{OW})		6.66	9.26	3.43	2.14	6.73	7.78	

* Cell-150 signify oxidized cellulose fibers with carboxylic content equal to 150 μmol/g

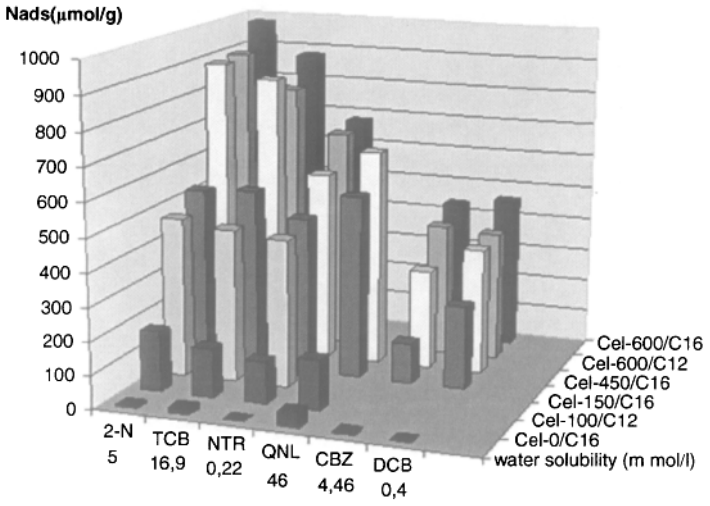


Figure 17.6 Histogram indicating maximum adsorbed amount of different organic solutes onto surfactant treated cellulose fibers. (Cel-150 means there is 150 µmol/g of COO- on the fibers surface).

continuous manner. To overcome this drawback, a chemical anchoring of the aliphatic chains to the cellulose surface is necessary.

17.4.2 Different Strategies of Surface Chemical Modification of Cellulose Fibres

Different chemical modification strategies were adopted in order to ensure a reproducible adsorption capacity of the cellulosic fibres independently of their origin, and to extend the adsorption potential toward a large range of organic pollutants. All of these reported modifications were aimed at grafting linear aliphatic chains with a length in the range of 6–18 carbons. The ensuing grafted hydrocarbon structure acts as a hydrophobic reservoir miming the aggregative domains of the adsorbed surfactants [29, 30, 31]. A high grafting level was necessary to enhance the adsorption properties of the modified fibres.

17.4.2.1 Modified Cellulose Fibres via Heterogeneous Esterification

Modified cellulose fibres through esterification were among the first substrate used in the adsorption of organic pollutants [32]. Esterification reaction has been carried in heterogeneous conditions by grafting octanoic anhydride onto the accessible cellulose hydroxyl groups. In order to increase their accessibility, cellulose fibres were subjected to a solvent exchange treatment, in which the fibres were dispersed in a series of organic solvents with different polarity

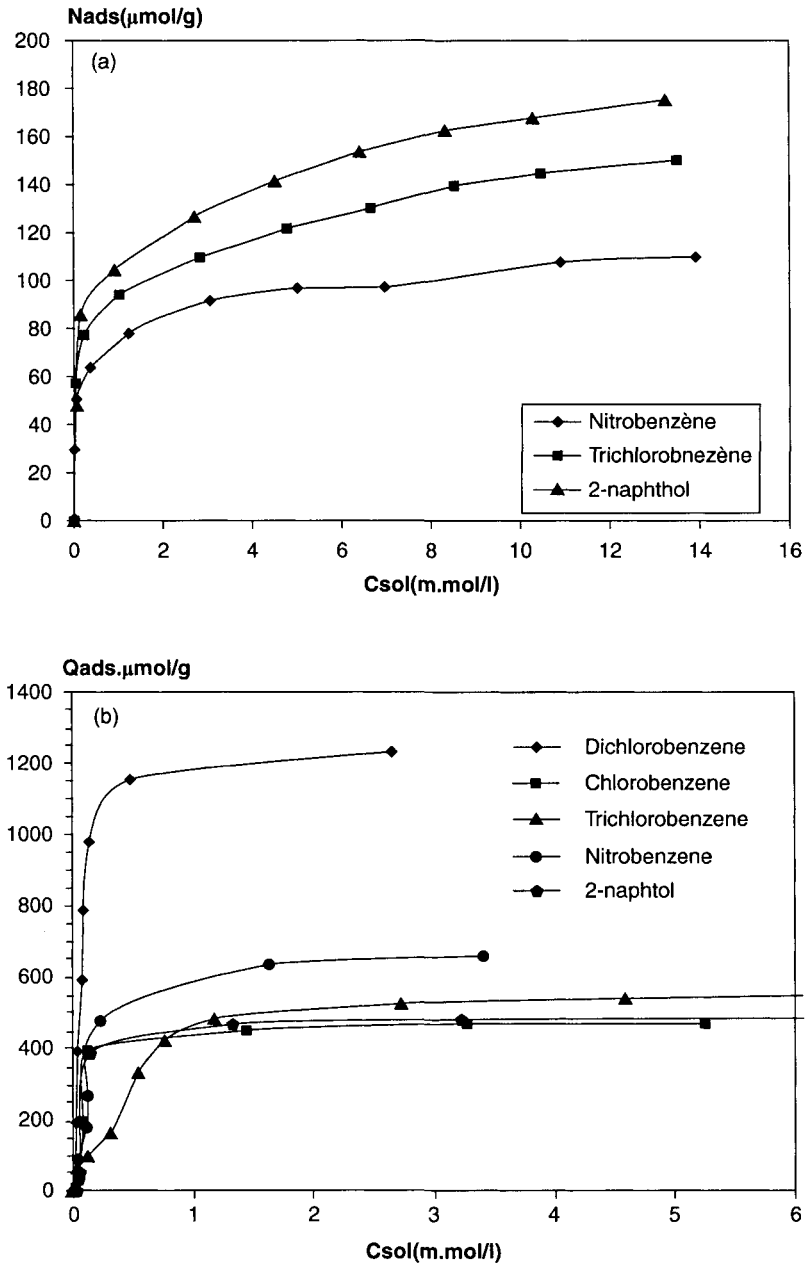


Figure 17.7 Adsorption isotherms of organic solutes on cellulose fibers treated with hexadecyammonium brimide C16 (coverage ratio $\theta = 75\%$). (a) virgin cellulose Cel-0, (b) oxidized cellulose Cel-600[25].

degrees going from the most polar solvent to the least. The different steps are depicted in Figures 17.8.

The occurrence of the grafting reaction at a high level via esterification was confirmed by FTIR analysis, showing an intense carbonyl band around

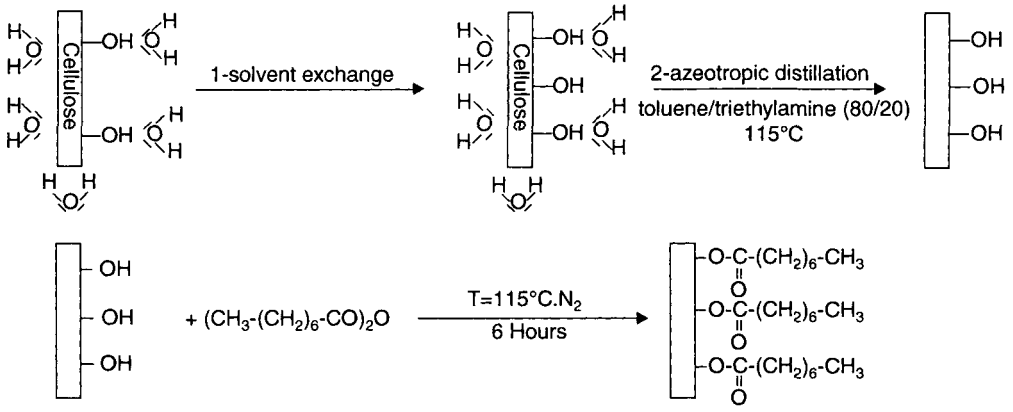


Figure 17.8 Mechanism of the cellulose esterification with octyl anhydride.

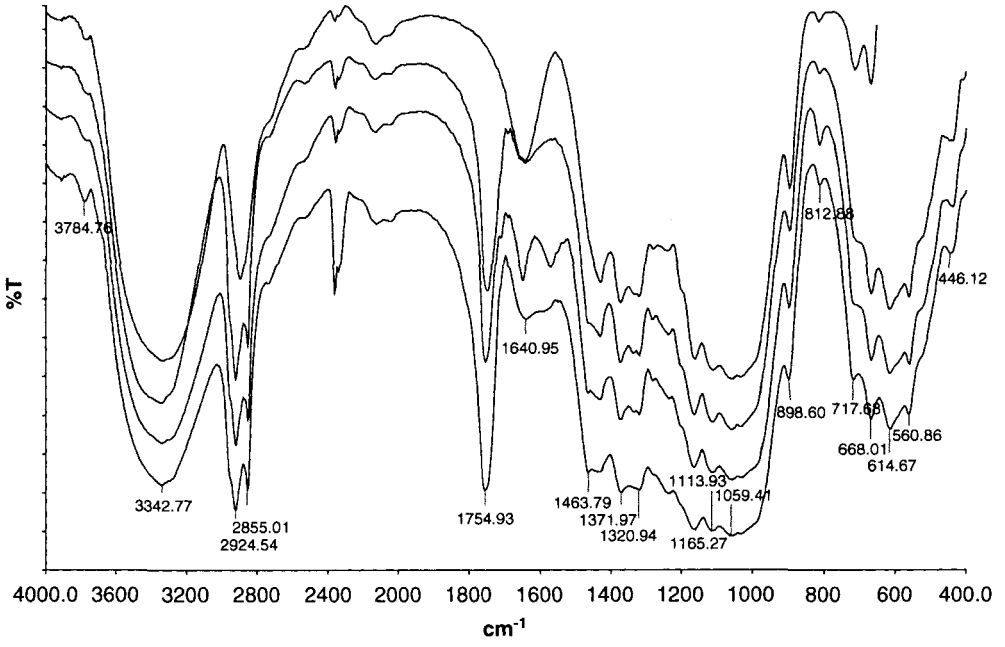


Figure 17.9 IRFT spectra of cellulose fibres after acylation with octanoic anhydride at different substitution degree[50] (DS), (a) pristine celulose, (b) Cell-DS:0.15), (c) Cell-DS:0.26, (d) Cell-DS:0.34.

1750 cm^{-1} (Figure 17.9) and by solid state ^{13}C NMR analysis (Figure 17.10). The latter clearly evidenced the presence of the aliphatic moiety with the emergence of new peaks at 12–40 ppm assigned to methylene carbon in the acyl moiety. The peaks of the cellulose backbone between 55–110 ppm did not undergo significant change after esterification, confirming the limitation of the esterification reaction only to the surface layer. Moreover, X-ray analysis confirmed that neither the structure of cellulose I nor the crystallinity degree was affected with the acylation modification.

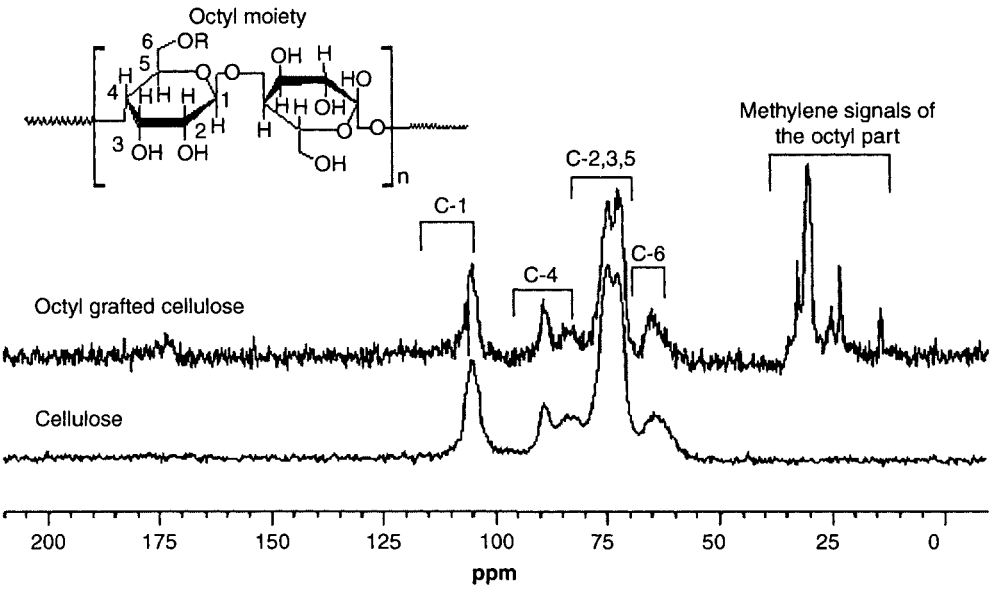


Figure 17.10 CP-MAS 13C NMR spectra of original cellulose fibres and the modified one after esterification with octanoic anhydride at a DS = 0.25[31].

Adsorption measurement carried on different organic compound revealed a significant enhancement in the ability of the cellulose substrate to trap dissolved organic compounds from water after modification. The adsorption capacity which does not exceed 40 μmol g^{-1} is raised up to 400 μmol g^{-1} after the grafting alkyl ester (Figure 17.11). The length of the appended organic moiety was shown to be an important parameter ruling the adsorption capacity of the modified fibres; the longer the hydrocarbon chain length of the acyl radical, the higher the maximum adsorption capacity of the substrate. The adsorption capacity seems to be more favoured for the less soluble compound, but no clear tendency could be established between the adsorption coefficient and the solubility.

A direct correlation between the grafting level of the modified fibres and the adsorption capacity was clearly established as shown in Figure 17.12. The higher the grafting level, the more important is the maximum adsorbed amount. However, the enhancement in the retention capacity is accompanied by an extension in the adsorption equilibrium kinetic being attained within 0.5 up to 3h depending on the length of the alkyl moiety and its grafting degree as shown in Figure 17.13.

The adsorption isotherms were fitted with the Langmuir model, from which the adsorption equilibrium constant K, as well as the maximum concentration of the solute uptake are established. The substrates can be easily recycled permanently by washing with ethanol or acetone without losing their adsorption capacity. This finding is one of the greatest advantages of such modified cellulose fibres compared to other much more conventional adsorbents such

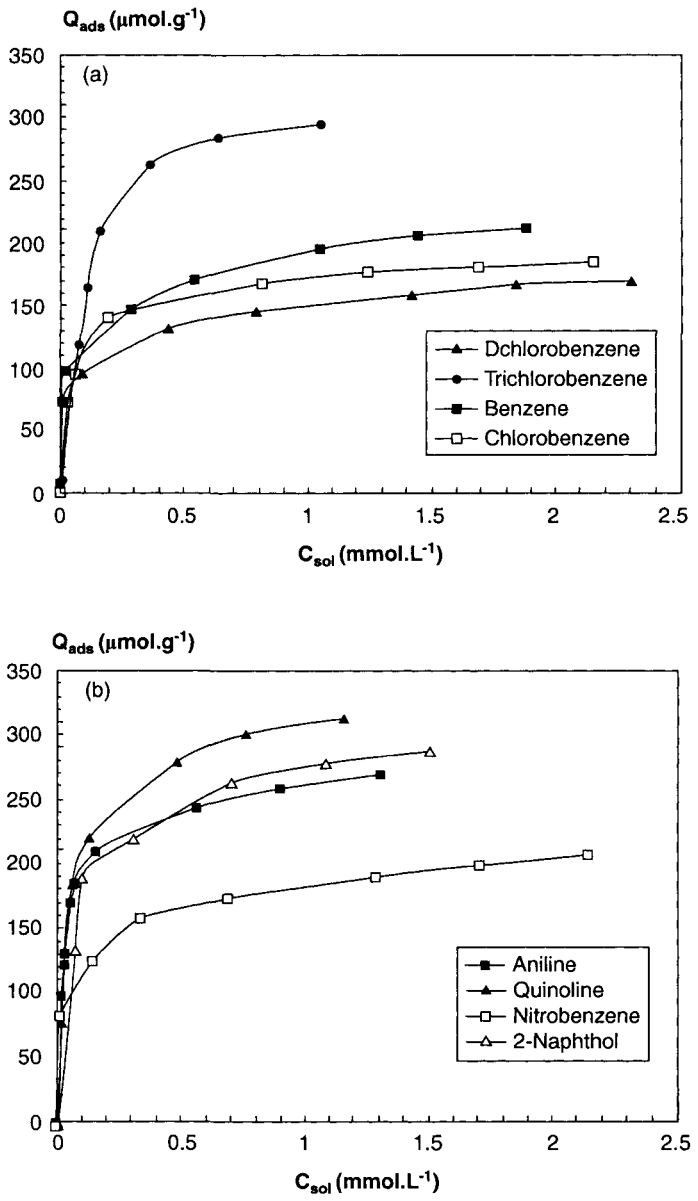


Figure 17.11 Equilibrium adsorption isotherms of different organic solutes onto (a), (b) modified cellulose fibres via esterification with octyl anhydride, and (c) virgin fibres, as a function of the equilibrium solute concentration at pH 6.5–7 and room temperature[31]. (reproduced from reference [51]). *(Continued)*

as activated carbon or zeolites, which require an energetically costly treatment and lead inevitably to a more pronounced loss of adsorption activity.

The adsorption properties of the modified cellulose fibres were also investigated under continuous condition using a filtration column filled with cellulose fibres modified by esterification with octanoic anhydride. The ratio (Cs/C0) of

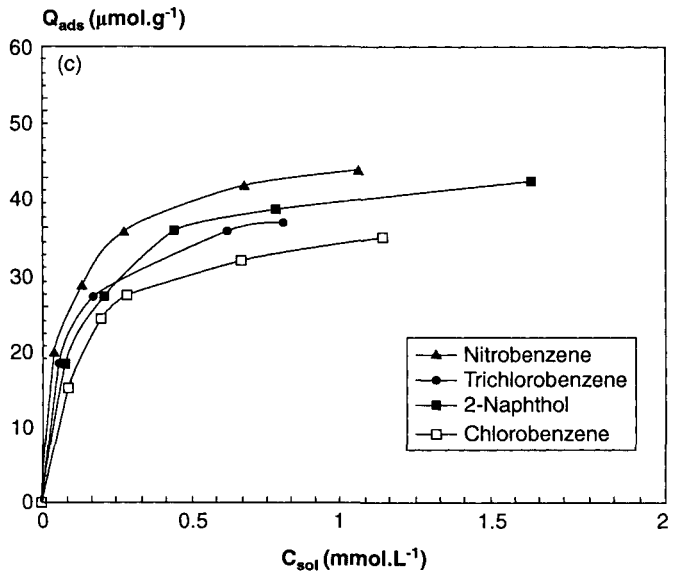


Figure 17.11 (cont.) Equilibrium adsorption isotherms of different organic solutes onto (a) modified cellulose fibres, and (b) virgin fibres, as a function of the equilibrium solute concentration at pH 6.5–7 and room temperature[31].

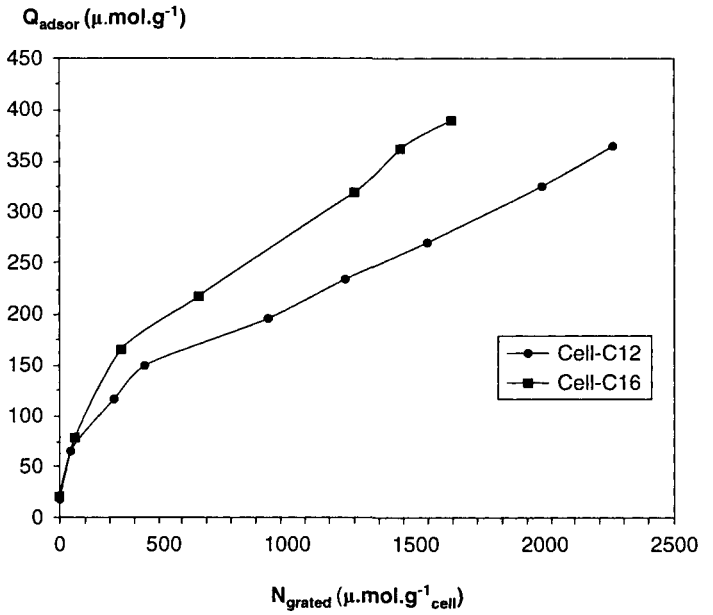


Figure 17.12 Evolution of the adsorption capacity vs. the acylation degree for Cell-C12 and Cell-C16; solute; 2-Naphthol, pH 6.5 –7: (*Cell-C12 and Cell-C16 refers to cellulose fibers grafted with dodecycl and hexadecyclanhydride acid respectively)*[23].

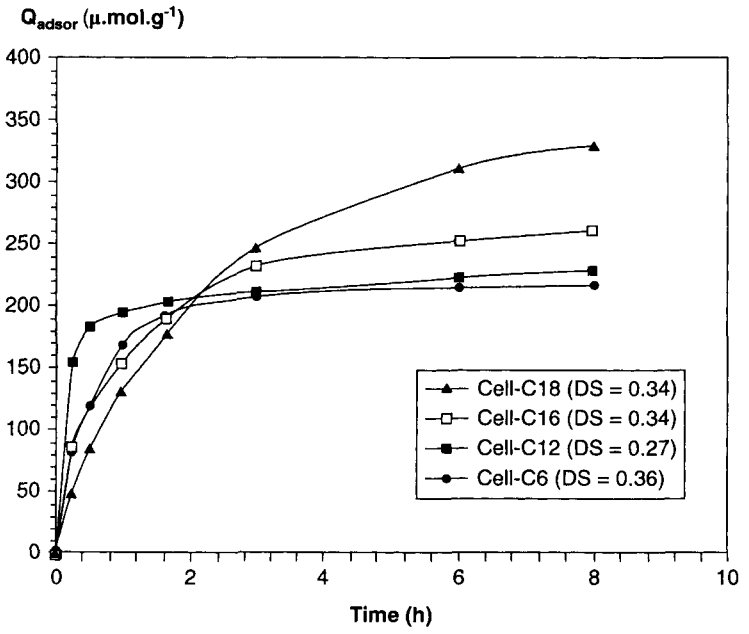


Figure 17.13 Kinetic data for 2-Naphthol adsorption onto Cell-C6, Cell-C12, Cell-C16 and Cell-C18: acylated level: 1200 –1400 µmol g^{-1}, solute concentration:400 µmol L^{-1} [23].

the effluent concentration Cs to the input concentration C0 was plotted against time to obtain the breakthrough curve at a constant flow rate. High removal efficiency, exceeding 95% was ensured as the adsorbed amount is lower than 75% of the maximum adsorption capacity. The effect of operating conditions: flow rate, feed concentration, adsorbed bed height, and temperature were explored, from which the most relevant conclusions can be summarized as follows:

- The breakthrough time is dependent on the flow rate of the solution, the residence time, and initial contaminant concentration.
- The higher bed length of the column, the longer the breakthrough time is.
- The increase of the adsorption temperature leads to an increase of the adsorption capacity.
- The ionic strength of the solution does not affect the adsorption capacity of the modified fibres up to 10^{-3} mol l^{-1}.

The regeneration of saturated modified cellulose fibres has been carried out using water/ethanol solution. The new adsorbent can be regenerated easily and reused for multiple cycles of treatment without any reduction of its adsorption capacity.

17.4.2.2 Chemical Modification Using a Diisocyanate

Isocyanate chemistry was also explored in order to graft long alkyl chains on the cellulose fibres and use the modified fibres as sorbent for organic pollutant

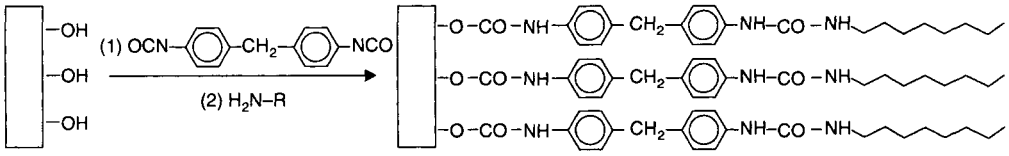


Figure 17.14 Mechanism of the cellulose grafting with MDI approach[29].

dissolved in water [29]. Compared to the ester group, the urethane linkage provides many advantages, namely: (i) relatively high reaction rates, (ii) the absence of elimination products and (iii) the chemical stability of the urethane moiety. The grafting reaction was carried out in two stages according to Figure 17.14; in the first one a difunctional isocyanate was appended on cellulose fibres while leaving the second NCO available. In the next step, a linear aliphatic amine was reacted with the isocyanate modified fibres in suspension of toluene/dimethylformamide (60/40 vol %). Different lengths of aliphatic amine ranging from C6 to C16 were tested. Given the high sensitivity of isocyanate to water, all the reactions were carried out under azeotropic distillation using Toluene/dimethylforamide (60/40 vol%) mixture to remove water adsorbed on cellulose fibres.

Evidence of the condensation reaction of the isocyanate functions with the OH groups of cellulose was supported by FTIR (bands at 1712 and 1640 cm^{-1} associated with CO and NH of the urethane, 1630–1500 cm^{-1} associated with CO and NH of urea, and bands at 2850–2900 cm^{-1} relative to aliphatic CH moieties of chain aliphatic). This evidence was also often corroborated by ^{13}C CP-MAS NMR with the emergence of the typical peaks at 12–40 ppm assigned to methylene carbon of the anchored hydrocarbon chain, peaks at 125–136 ppm assigned to aromatic rings of MDI, and peaks at 157.46 ppm related to the carbamate functions. An example of ^{13}C CP-MAS NMR of the is shown in Figure 17.15.

The adsorption ability of the modified substrate was investigated using five aromatic organic compounds differing in their structure and polarity (Table 17.3) as a model organic pollutant. The adsorption isotherm of on Cel-MDI-C8 revealed that the fibre modification greatly enhanced the aptitude of the substrate to uptake dissolved organic compound from water. The adsorption capacity grows from 4-8 $mg \cdot g^{-1}$ for the virgin fibres towards higher values ranging from 30 $mg \cdot g^{-1}$ up to 70 $mg \cdot g^{-1}$ after fibre modification. Likewise, the adsorption capacity is amplified with the grafting degree and with the increase in the length of the grafted alkyl chain.

17.4.2.3 Chemical Modification through Activation with N,N'-carbodiimidazole (CDI)

One of the most promising approaches regarding the surface modification of cellulose fibres toward the preparation of a sorbent based on cellulose substrate for the uptake of organic pollutant, calls upon CDI as an activation agent. CDI was first used in 1960 by Paul and Anderson [33] as a peptide coupling by

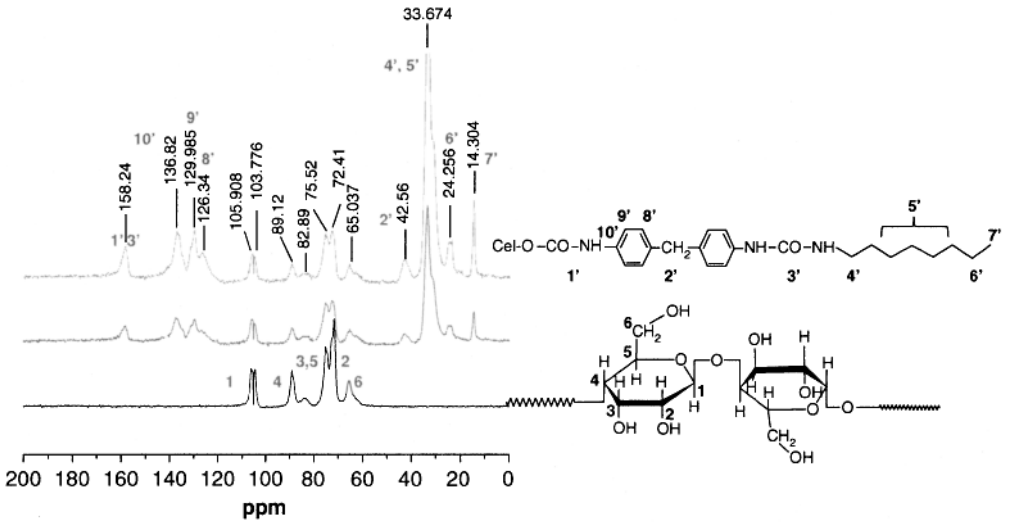


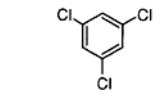
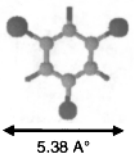
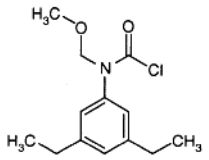
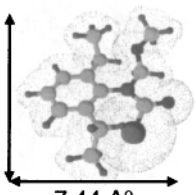
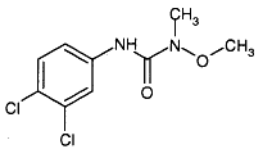
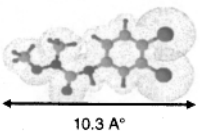
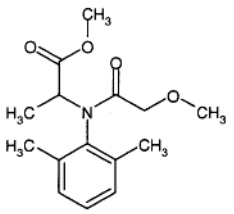
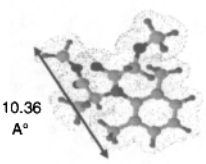
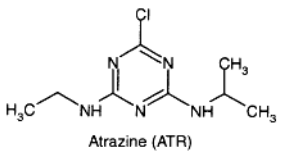
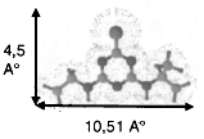
Figure 17.15 CP-MAS NMR spectra of the original cellulose fibres (a) and the modified sample Cel-MDI-C8 (b)[30].

Table 17.3 Structures of tested organic solutes and herbicides, their water solubility and Ln(K_{ow}) values[28].

Organic solutes & herbicides			
Structure & abbreviation	**3D structure**	**Water solubility**	**Ln(K_{ow})**
2-naphthol (2-N)	7.83 A° / 5 A°	5 mmol.L^{-1}	6.66
Nitrobenzene (NTR)	4.33 A° / 6 A°	16.9 mmol.L^{-1}	3.43
Chlorobenzene (CBZ)	5.6 A°	4.46 mmol.L^{-1}	6.73
Dichlorobenzene (DCBZ)	5.38 A°	0.4 mmol.L^{-1}	7.78

(Continued)

Table 17.3 (cont.) Structures of tested organic solutes and herbicides, their water solubility and Ln(K_{ow}) values[28].

Organic solutes & herbicides			
Structure & abbreviation	**3D structure**	**Water solubility**	**Ln(K_{ow})**
 Trichlorobenzene (TCBZ)	 5.38 A°	0.22 mmol.L^{-1}	9.26
 Alachlor (ACH)	 7,44 A°	240 mg.L^{-1}	2.9
 Linuron (LNR)	 10.3 A°	81 mg.L^{-1}	3.00
 Metalaxyl (MTX)	 10.36 A°	8400 mg.L^{-1}	1.75
 Atrazine (ATR)	 4,5 A° 10,51 A°	28 mg.L^{-1}	2,7

activation of aliphatic carboxylic acids to form imidazole carboxylic ester, and enabling subsequent reaction with amines. Later, CDI was used in the selective synthesis of amides [34], carbamates [35] and also in the synthesis of a number of pharmaceutical products, e.g. sildenafil [36] and sampatrilat [37]. In the field of carbohydrate chemistry, Heinze and coworkers have used CDI as an activator for carboxylic acid to prepare under homogeneous condition a wide variety of cellulose ester derivatives bearing unsaturated, chiral, crownether, cyclodextrin structures, and dendrons [38, 39, 40]. The particularity of CDI is its ability

to react with alcohol, carboxylic acid, and amine groups giving rise to reactive carbonyl imidazole intermediates that are more easily handled and may be isolated, if necessary. The ensuing carbonyl imidazole could subsequently undergo selective reactions with primary amines or primary alcohols to form amide, carbonate, or ester derivative [37, 38]. The advantages of this method include the mild reaction conditions which minimize secondary reaction, the lack of formation of amine hydrochloride when using acid chlorides, and the avoidance of lengthy purification stages. Moreover, imidazole, the by-product obtained both when CDI reacts with hydroxyl and carboxylic acid, and after the reaction of carbonyl imidazole with alcohol or amine, is easily removed from the reaction mixture by an acidic wash. These advantages, coupled with the relatively low cost of CDI, render this method an attractive alternative to the carbodiimide-based reagents such as N,N-dicyclohexylcarbodiimide (DCC) [41] or acide chloride.

Adopting the CDI approach under heterogeneous condition, different sequences of modification have been accomplished using difunctional or trifunctional amines [32, 33] (Figure 17.16). It was shown that the reaction on the CDI activated cellulose substrate proceeds through the condensation of a single amine group leaving the second terminal amine function available for further reaction. This latter one could again be activated by CDI to generate carboxamide imidazole and undergo condensation with aliphatic amine or diamine. This strategy enables wide latitude of target surface functionalisation and opens a novel way of achieving molecular architecture on cellulose substrate. Examples of molecular architecture appended on cellulose fibres are depicted in Table 17.4.

The heterogeneous condensation was carried on in two steps; during the first step, hydroxyl groups of cellulose surface are activated with CDI at 60°C under nitrogen atmosphere for a period of 3 hours. The ensuing imidazolide is isolated and added to a solution of dry toluene containing amine, diamine, or triamine. For further functionalisation, the ensuing fibres were activated again with CDI followed by the interaction with an aliphatic amine or carboxylic

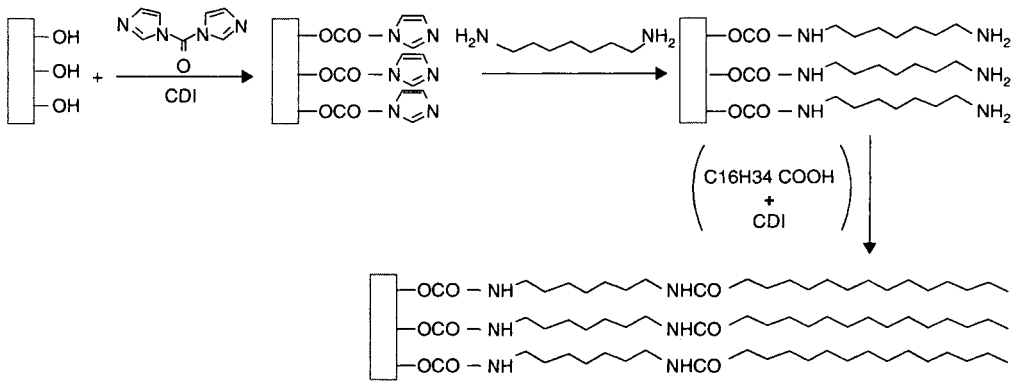
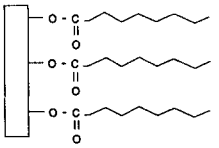
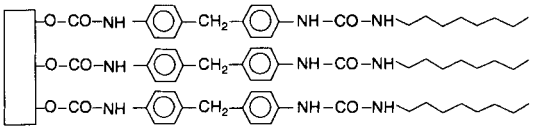
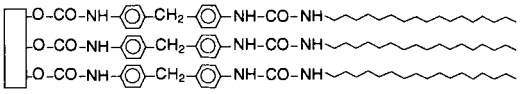
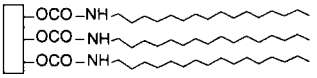
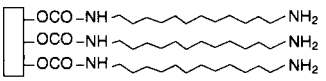
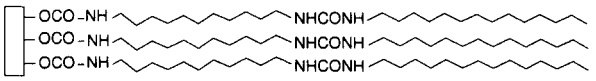
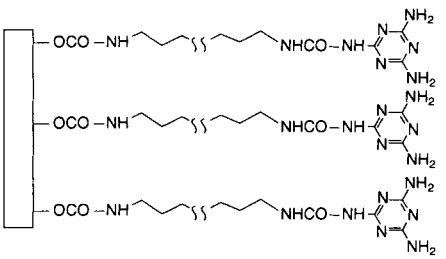


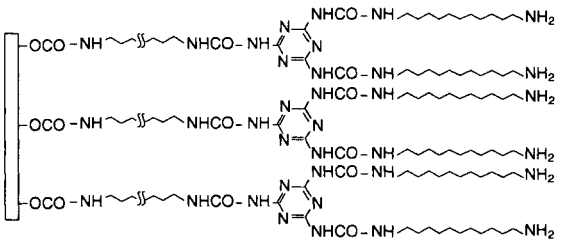
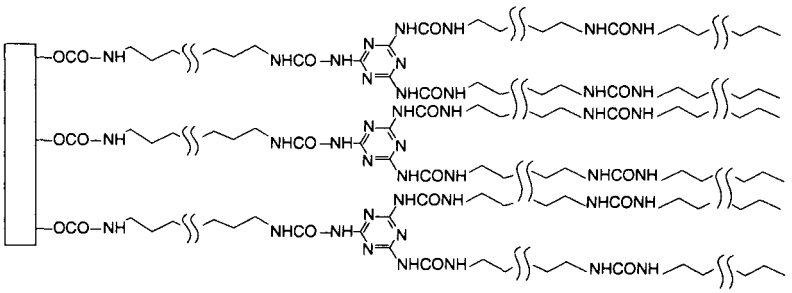
Figure 17.16 Mechanism of the cellulose grafting with CDI approach[28].

Table 17.4 chemical structures of the appended moiety on the different modified cellulose fibers.

Modified fibers structures and abbreviation	Numbering
 Cel-OCO-C8	(I)
 Cel-MDI-NHC8	(II)
 Cel-MDI-NHC16	(III)
 Cel-CDI-NHC16	(IV)
 Cel-CDI-NHC12NH2	(V)
 Cel-CDI-NHC12NH-CDI-NHC16	(VI)
 Cel-CDI-NHC12NH-CDI-MM	(VII)

(Continued)

Table 17.4 (cont.) chemical structures of the appended moiety on the different modified cellulose fibers.

Modified fibers structures and abbreviation	Numbering
	(VIII)
	(IX)

acid. Finally, the recovered product was purified by soxhlet extraction with toluene for 48 h, and dried at 40°C for 24 h [33].

A multitude of characterisation techniques were used to evidence the occurrence of the different reaction sequences; Fourier transform infrared spectroscopy (FTIRS), solid NMR spectroscopy (C-PMAS) (Figure 17.17), contact angle measurements, and X-ray photoelectron spectroscopy (XPS) (Figure 17.18).

17.5 Adsorption Properties of Modified Cellulose Fibres

The ability of the modified fibres to uptake organic compounds from aqueous solution was investigated under batch and continuous conditions. The adsorption isotherms of different organic solutes, previously dissolved in an aqueous suspension of modified cellulose fibres, were established. Six aromatic organic compounds differing by their structure and polarity (Table 17.3) were selected as models in order to investigate the adsorption ability of the modified fibres and to assess how the chemical modification of delignified cellulose fibres affects the adsorption capacity of the material. The adsorption isotherms of the organic solutes on sample (IV) and (VI) (see table 17.4) are

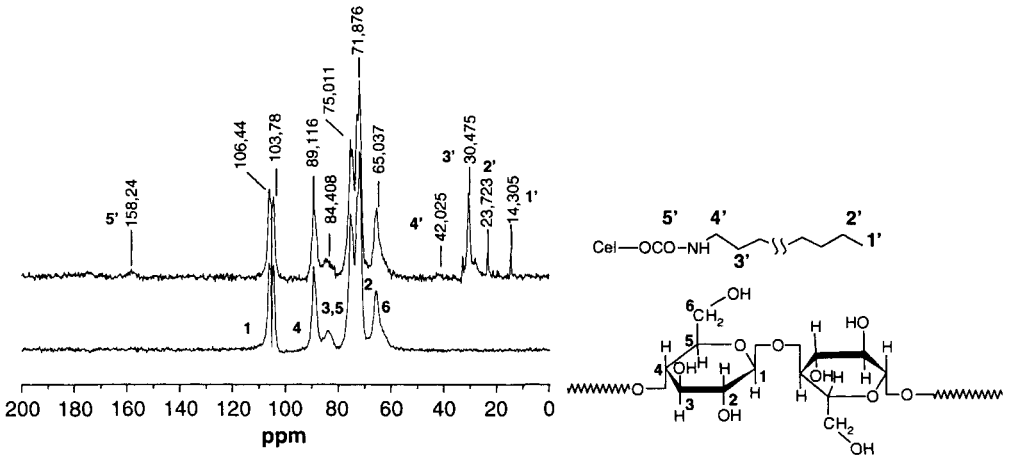


Figure 17.17 NMR spectra of virgin cellulose and the modified fibers by an aliphatic amine in C16 (sample (IV))[30].

shown in Figure 17.19. For the different organic compounds, the adsorption increased rapidly at low equilibrium solute concentration and then levelled off at a plateau beyond a concentration ranging from 0.5–1 mmolL^{-1}. The adsorption capacities of the selected solutes on virgin and modified fibres are given in Table 17.5 from which the following remarks can be put forward:

i. The virgin fibres displayed low adsorption capacity toward dissolved organic solutes, being in the range of 3–7 mg.g^{-1} which is within the limit of other reported lignocellulosic material derived from the biomass [42].
ii. The chemical modification greatly enhanced the adsorption ability of the organic solutes by the fibres, which ranged from 45–170 mg.g^{-1} depending on the organic solute structures.
iii. The adsorption capacity depended on the chemical structure of the organic compound. No clear dependence, neither on the water solubility nor on the K_{ow} coefficient (the partition coefficient of the solute between the octanol and water phases) was established, suggesting that the hydrophobicity character of the organic compounds and their water solubility are not the only parameters governing the adsorption process. Other factors, such as the hydrodynamic volume, the shape of the molecule, and the interaction potential between the adsorbent and adsorbate, are likely to play an important role.

Luminescence measurements had shown that in addition to Wan der Waals interactions, the adsorption of polar molecules on the modified cellulose fibres involves polar and hydrogen bonding with the cellulose AGUs [34, 35].

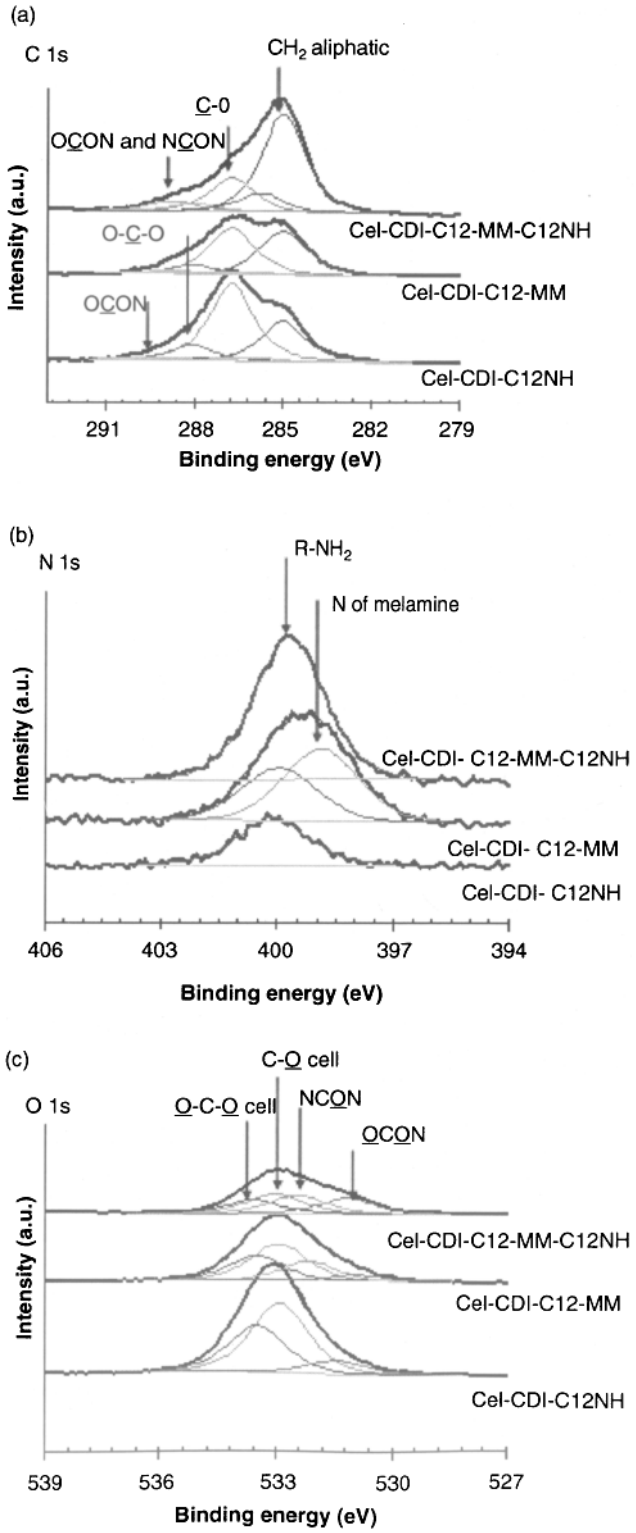


Figure 17.18 XPS regions (a) C 1s, (b) N 1s and (c) O 1s of (from bottom to top) (V); (VII) and (VIII); (where MM: melamine ring)[30].

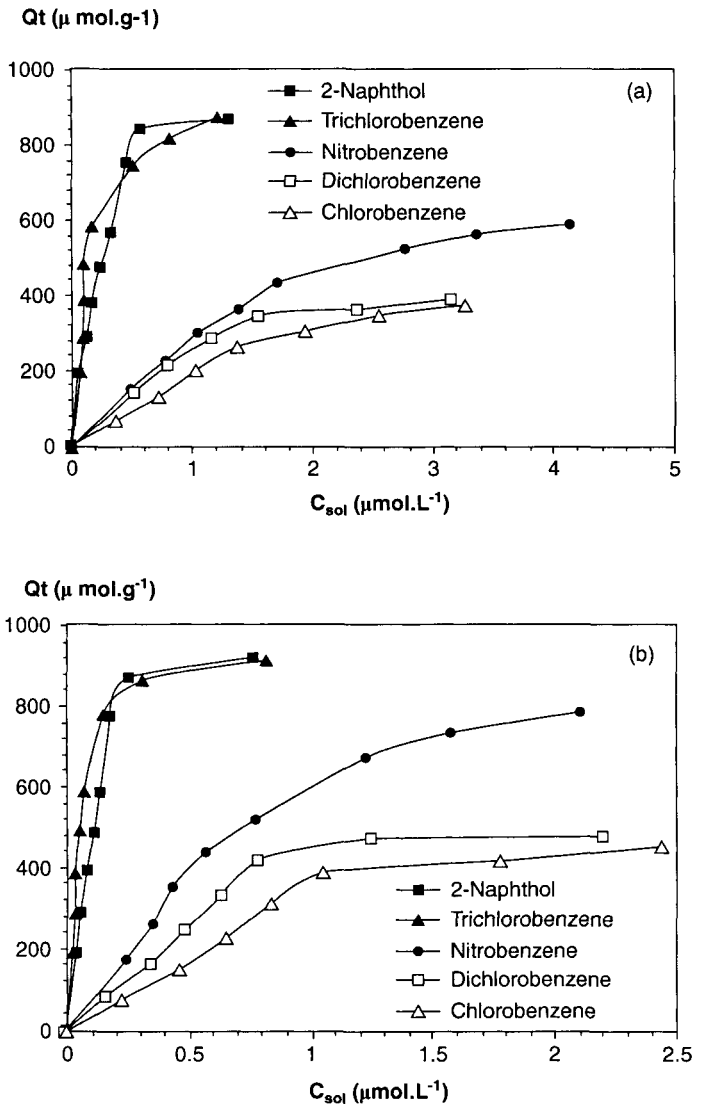


Figure 17.19 Adsorption isotherms of organic solutes on (a): sample (IV) and (b): sample (VI)[29].

iv. The extension of the length of the grafted moiety did not contribute to enhance significantly the adsorption capacity of the organic solutes. Only a moderate raise of 10–15% in the maximum adsorption level was noted by increasing the anchored alkyl chain from 16– 28 methylene groups, i.e., (IV) against (VI).

17.5.1 Adsorption of Herbicides

The ability of modified cellulose substrate for the uptake of herbicides was also investigated using alachlor (ACH), metalaxyl (MTX), linuron (LNR), and

Table 17.5 Maximum adsorbed amounts ($mg.g^{-1}$) of adsorbed organic solutes onto chemically modified cellulose fibers[28].

Substrate	Ref	2-N	NTR	TCB	DCB	CBZ
Virgin cellulose	32	6	5	7	5	4
Sample (I)	32	40	26	53	25	20
(II)[a]	34	38	15	42		
(II)[b]	34	63	23	68		
(II)[c]	34	73	29	77		
(II)[d]	34	75	28	78		
(III)	34	72	29	77		
(IV)	33	125	68	162	55	45
(VI)	33	134	97	168	72	52

(II)[d] had a grafting level higher than (II)[c], higher than (II)[b], higher than (II)[a].

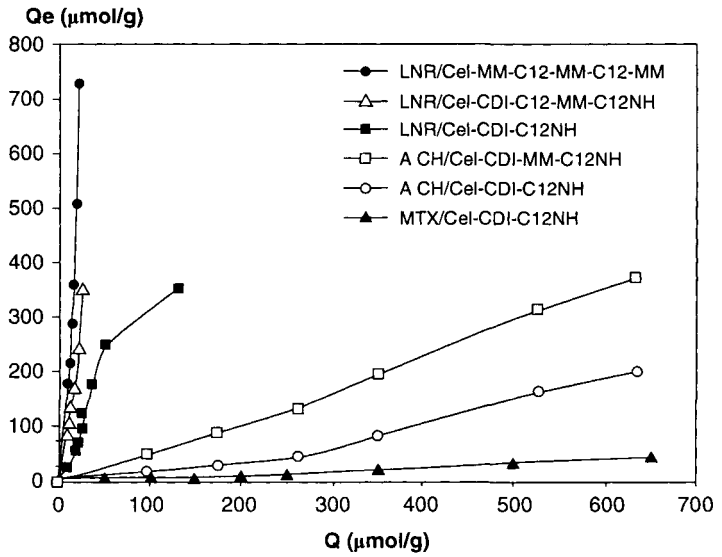


Figure 17.20 Adsorption isotherms of herbicides on different modified cellulose fibers[28].

atrazine (ATR) as shown in Figure 17.20. The modified fibres were revealed to be efficient in trapping herbicide likely to be present in water. However, the adsorption efficiency depended on the chemical structure of the pesticides as well as on the nature of the cellulose modification as shown in Table 17.6. The

Table 17.6 Maximum adsorbed amounts (μmol.g^{-1}) of adsorbed herbicides onto chemically modified cellulose fibers.

	Linuron	Alachlor	Metalaxyl	Atrazine
(III)	135	50	20	–
(IV)	150	75	50	–
(VI)	350	200	156	–
(V)	200	125	76	–
(VII)	560	386	–	23
(VIII)	590	358	110	28
(IX)	730	480	–	–

presence of polar groups, likely to set up hydrogen bonding with the substrate, promotes the adsorption of the herbicide. Likewise, the herbicide with a planar structure such as LNR displayed higher adsorption ability as a result of their intercalation inside the domains formed by the grafted chains. A schematic illustration of the adsorption mechanism of herbicides onto sample (VIII) substrate was proposed as depicted on Figure 17.21.

17.6 Adsorption Isotherm Modelisation

The Langmuir and Freundlich models were adopted to fit the experimental adsorption isotherms. The former model was developed for well-defined localized adsorption sites having the same adsorption energy independent of surface coverage, and with no interaction between adsorbed molecules. In this model:

$$q_e = \frac{Q_{\max} K_L C_e}{1 + K_L C_e} \tag{17.1}$$

where q_e is the amount of solute adsorbed at equilibrium per unit weight of adsorbent (mg.g^{-1}), C_e is the equilibrium concentration of solute in the bulk solution (mmol.L^{-1}), Q_{max} is the maximum adsorption capacity (mg.g^{-1}), and K_L is the constant related to the free energy of adsorption. The linearised form of the equation can be written as follows:

$$\frac{C_e}{q_e} = \frac{1}{K_L Q_{\max}} + \frac{C_e}{Q_{\max}} \tag{17.2}$$

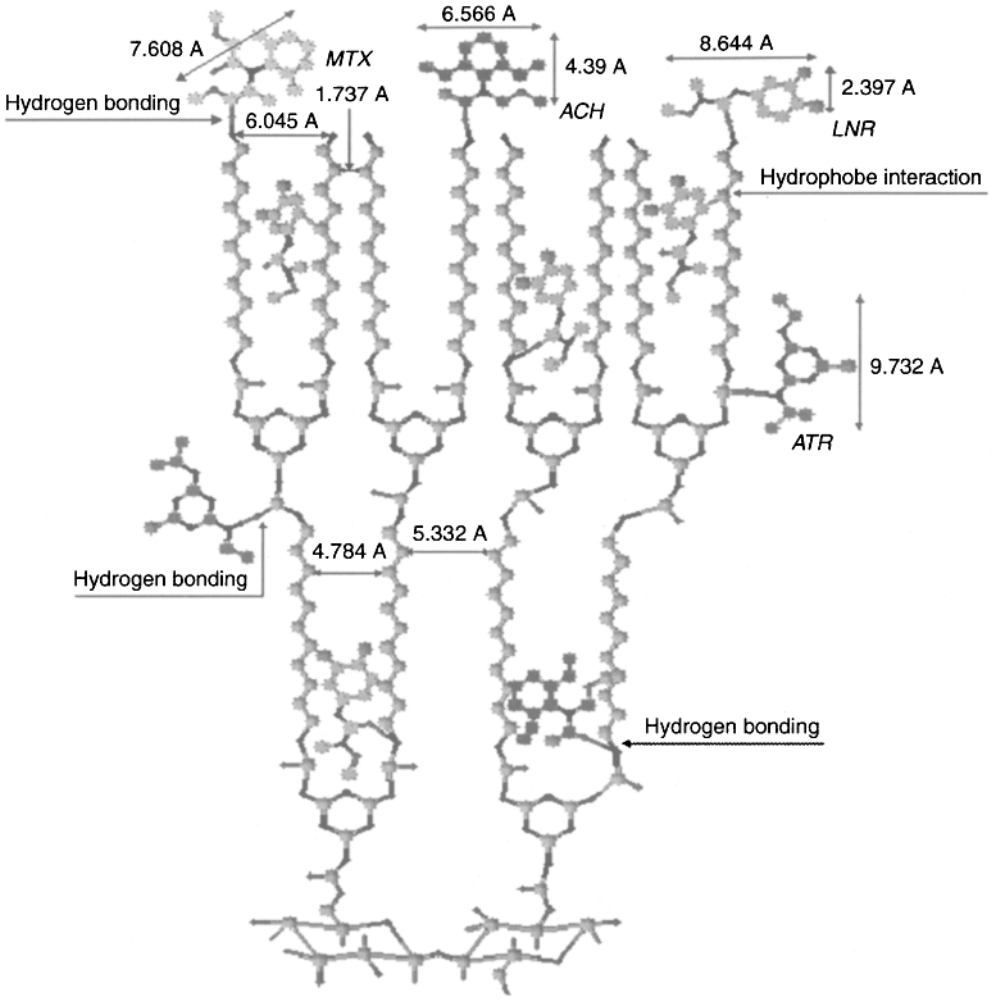


Figure 17.21 Schematic illustration of the adsorption mechanism of herbicides on modified cellulose by CDI activation[28].

From the data of C_e/q_e *versus* C_e, K_L and Q_{max} can be determined from the slope and intercept.

The Freundlich isotherm is an empirical one appropriate for the adsorption processes where non-uniformity of the surface of adsorbent is expected, and is given by:

$$q_e = K_F C_e^{1/n} \tag{17.3}$$

where K_F and n (dimensionless) are constants incorporating all factors affecting the adsorption process such as interaction energy and adsorption effectiveness, respectively. This equation can be linearized as follows:

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \tag{17.4}$$

The applicability of the isotherm equations was compared on the basis of correlation coefficient, R^2. Table 17.7 gathers together results deduced from Langmuir and Freundlich isotherms for a series of modified cellulose fibres.

17.7 Thermodynamic Parameters

The thermodynamic parameters such as the standard Gibbs free energy change (ΔG^0), enthalpy change (ΔH^0), and entropy change (ΔS^0) were evaluated for each adsorption system [32, 33]. The Gibbs free energy change of the process is related to the equilibrium constant by the equation:

$$\Delta G° = -RT \ln Kc \tag{17.5}$$

The equilibrium constant K_c was defined as $K_c = \frac{F_e}{1 - F_e}$ where F_e is the fraction of adsorbed solute at equilibrium.

The thermodynamic parameters for the adsorption process, ΔH^0 and ΔS^0, were evaluated using the following equation:

$$\ln K_c = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \tag{17.6}$$

The values of ΔG^0 and of ΔH^0 and ΔS^0 calculated from the slope and intercept of linear regression of $\ln K_c$ versus $(1/T)$, at different coverage level are given in Table 17.8. A negative value of ΔG^0 at all temperatures was found indicating a spontaneous adsorption of the organic compound. For all the studied solute, the negative value of ΔH^0 is indicative of an exothermic adsorption process. The value of ΔH^0 was found to be in the range -30—55 kJ.mol^{-1} suggestive of an adsorption driven by dispersive and polar Van der Waals interactions between the grafted chain moiety and the solute molecules. Furthermore, a negative value of ΔS^0 was also reported in agreement with the restriction of the motion and the randomness degree.

17.8 Adsorption Kinetic Modelling

The kinetic analysis of the adsorption process using cellulose fibres modified by grafting alkyl chains according to one of the previous methods, could be fitted with the second-order kinetic model [43] expressed as:

$$\frac{t}{q_t} = \frac{1}{(k_2 q_e)^2} + \frac{t}{q_e} \tag{17.7}$$

where k_2 (mg μmol^{-1} min^{-1}) is the rate constant of second order adsorption.

Table 17.7 Langmuir and Freudlich constants of the adsorption isotherms of different organic solutes on modified cellulose fibres[32,35].

		2-N	CBZ	DCBZ	TCBZ	NTR
Sample (I)						
Langm.	$C_{max(L)}$ (mg.g^{-1})	41	21	25	54	25
	C_{max}(mg.g^{-1})	40	20	25	54	26
	K_L(L.mol^{-1})	8.75	14	11	8.9	1
	R^2	0.996	0.996	0.996	0.993	0.995
Cel-CDI-C16 (IV)						
Langm.	$C_{max(L)}$ (mg.g^{-1})	133	94	93	182	115
	C_{max}(mg.g^{-1})	126	42	57	160	73
	K_L(L.mol^{-1})	3.8	0.278	0.59	6.12	1
	R^2	0.97	0.8474	0.9133	0.992	0.9833
Freud.	1/n	0.5645	0.7988	0.6969	0.4166	0.6269
	K_F(mg.g^{-1})	9.89	1.72	2.17	9.42	2.72
	R^2	0.9124	0.9581	0.9205	0.8345	0.963
Cel-CDI-C12-C16 (VI)						
Langm.	$C_{max(L)}$ (mg. g^{-1})	162	95	107	197	165
	C_{max}(mg.g^{-1})	133	51	71	167	99
	K_L(L.mol^{-1})	7.36	0.57	1.1	18.54	0.792
	R^2	0.9681	0.8231	0.8871	0.9971	0.9426
Freud.	1/n	0.5477	0.76	0.6935	0.3795	0.6734
	K_F(mg.g^{-1})	15.23	2.94	3.84	12.97	5.62
	R^2	0.8291	0.9149	0.8916	0.8075	0.9398
Cel-MDI-C8 (II)						
Langm.	$C_{max(L)}$ (mg.g^{-1})	95	–	–	113	40
	C_{max}(mg.g^{-1})	111	–	–	82	29
	K_L(L.mol^{-1})	0.478	–	–	0.384	0.355
	R^2	0.9732	–	–	0.9931	0.98
Freud.	1/n	0.5585	–	–	0.5896	0.6063
	K_F(mg.g^{-1})	1.91	–	–	1.65	0.783
	R^2	0.9926	–	–	0.9902	0.934

(Continued)

Table 17.7 (cont.) Langmuir and Freudlich constants of of the adsorption isotherms of different organic solutes on modified cellulose fibres[32,35].

		2-N	CBZ	DCBZ	TCBZ	NTR
Cel-CDI-MM-C10-C16						
Langm.	$C_{max(L)}$ (mg.g^{-1})	158	80	100	218	156
	C_{max}(mg.g^{-1})	128	55	81	154	102
	K_L(L.mol^{-1})	4.34	1.195	3.04	1.705	1.21
	R^2	0.9435	0.8677	0.9792	0.8166	0.8463
Freud.	1/n	0.5478	0.6532	0.562	0.6282	0.62
	K_F(mg.g^{-1})	11.34	3.83	5.59	8.29	6.99
	R^2	0.7531	0.823	0.8755	0.7212	0.8033

Table 17.8 Thermodynamic parameters for the adsorption of 2-N and NTR on Cel-MDI-C16(1/0.7) and Cel-CDI-MM-C10-C16.

	ΔH^0 (KJ.mol^{-1})	ΔS^0 (J.K^{-1}.mol^{-1})	$\Delta G^0_{(298)}$ (KJ.mol^{-1})	$\Delta G^0_{(308)}$ (KJ.mol^{-1})	$\Delta G^0_{(318)}$ (KJ.mol^{-1})
Cel-CDI-MM-C10-C16 (NTR)					
1st level	–30	–24	–22.962	–22.74	–22.5
2nd level	–38	–51	–23.39	–22.5	–22.38
3rd level	–58	–117	–23.97	–22.038	–21.67
Sample (III) (2-N)					
1st level	–35	138	–5.9	–7.15	–8.7
2nd level	–28	114	–6.3	–7	–8.6
3rd level	–28	114	–6.3	–7.15	–8.6
Sample (III) (NTR)					
	–44	180	–5.9	–7.5	–9 .5

Owing to the particular structure of the substrate with a surface bearing a relatively high density of hydrocarbon chains protruding toward the continuous medium, the diffusion of sorbate inside these chains cannot be ignored, which may constitute the rate controlling step. This diffusion process could be described by Weber and Morris [44], which is based on Fick's Second Law [45].

$$q_t = k_D \sqrt{t} \tag{17.8}$$

where q_t is the sorbed concentration ($mg.g^{-1}$) at time t, and k_D is the internal diffusion parameter for intraparticle transport. In the presence of cellulose substrate modified by grafting only one type of alkyl chain, this analysis indicated that after several minutes of the experiment, where external mass transfer involving the movement of adsorbate molecules from the bulk of the solution towards the external surface of the adsorbent is the dominant process, the evolution of q_t *vs* $t^{0.5}$ could be fitted with two linear portions. On the other hand, when two alkyl chains connected through a polar amido linkage was grafted on the fibre's surface the plot could be linearised with three linear portions, consistent with an adsorption involving a multistep process. The first step in the adsorption process portion corresponds to the external surface adsorption or instantaneous adsorption stage. The second one involves the gradual adsorption stage, where the diffusion within the grafted hydrocarbon chains was rate-controlled. The third step corresponds to the final equilibrium stage [46].

17.9 Column Studies

Using a filtration column filled with cellulose fibres modified with alkyl chains, the adsorption properties of the modified fibres under dynamic conditions were investigated. The breakthrough curves at a constant flow rate were established for a large series of organic pollutant models, involving herbicides [29, 30]. A high retention efficiency of the dissolved organic compounds by the column exceeding 90% was observed since the adsorption capacity did not reach a critical level ranging from 70–80% of the maximum retention capacity. Up to this threshold, the retention efficiency decreased until the exhaustion of the column. An example of the breakthrough curves are shown in Figure 17.22.

17.10 Column Regeneration

The regeneration ability of an adsorbent and its re-use over a number of adsorption/desorption cycles is one of the most important requirements which determine, from an economical point of view, the potential exploitation of a material as an adsorbent. Investigation carried out on different aromatic organic pollutants and different herbicides showed a complete desorption of the trapped pollutant after elution with an organic solvent in which the adsorbed pollutant is highly soluble. Depending on the solute structure, the bed volume needed to strip off the adsorbed pollutant ranged from 3–10, which means that the amount of solvent to ensure complete desorption is not excessive. The regenerated column could be used in a multitude of adsorption-desorption cycles without any loss of the adsorption capacity.

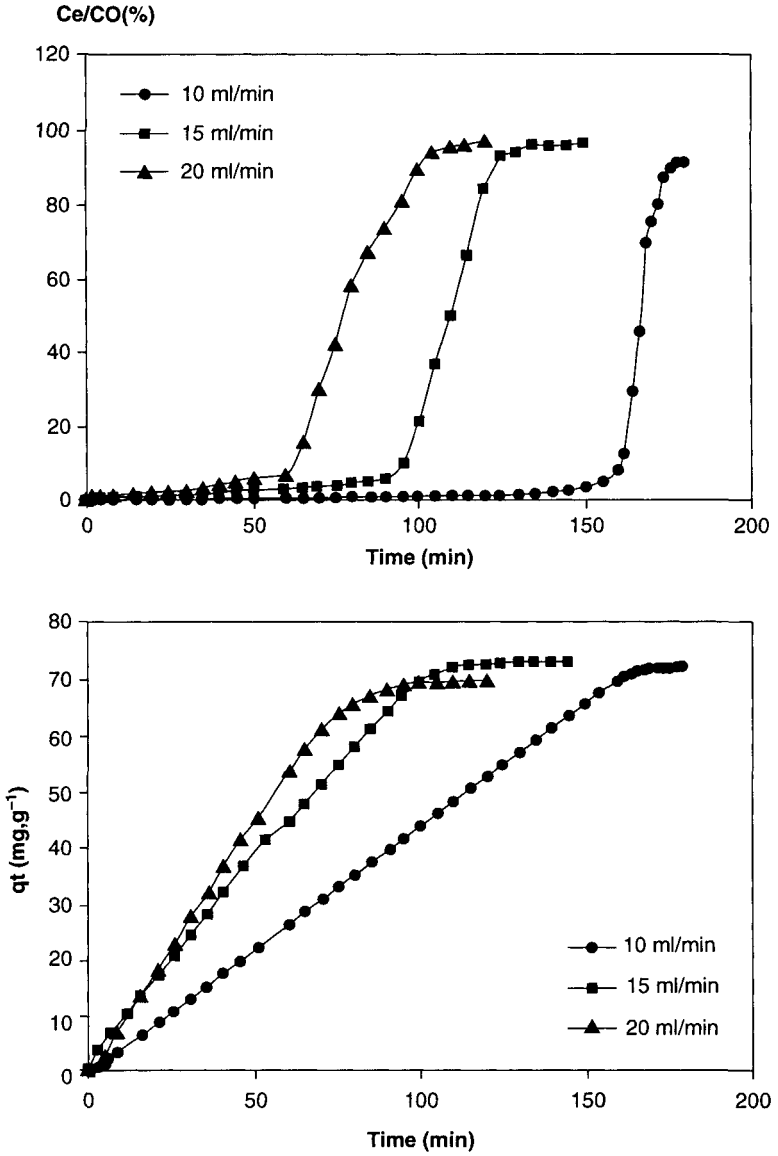


Figure 17.22 Effect of the feed flow rate on the breakthrough curves (a) and on the corresponding retained amount vs. time (b) of 2-naphthol uptake onto sample (III) under continuous flow (temperature: 20°C)[28].

17.11 Investigation of Adsorption Mechanisms by Laser Induced Luminescence

Using benzophenone (BZP) as probing hosts, laser induced luminescence was employed to get a deeper understanding regarding the mechanism involved

in the adsorption of organic pollutant with cellulose fibres grafted with alkyl chains. The $n \rightarrow \pi^*$ absorption transition of the probe is known to be very sensitive to the environment characteristics and also exhibits a photochemistry which depends on the host properties [47, 48, 49]. A comparison of the photochemical behaviour of BZP adsorbed onto 'normal' microcrystalline cellulose and a modified one was made. It was concluded that BZP is well entrapped within the modified cellulose polymer chains which provide a rigid environment to the guest molecule. Polar interaction and hydrogen bonding with hydroxyl groups amide or amine, also contribute to the adsorption process.

17.12 Conclusion

The main emphasis of this chapter dealt with the exploitation of natural lignocellulosic material and modified cellulose fibres as a sorbent for the uptake of dissolved organic pollutants from water. In this context, advantages of using lignocellulosic material as a sorbent rely on its abundance, its chemical stability, its renewable character, its porosity, and the possibility for it to carry on a multitude of chemical reactions based on the OH chemistry. Specific Highlight was accorded to modified cellulose fibres based on surface grafting of long hydrophobic chains liable to act either/both as a hydrophobic reservoir for water-insoluble organic molecules, or bearing functional groups likely to promote adsorption through hydrogen bonding. The chemical modification of the surface of the cellulose fibres has proven to be efficient to boost the adsorption capacity toward a wide range of organic pollutants, including herbicides and pesticides. The ensuing modified substrate can successfully compete in term of adsorption capacity with commercial sorbent such as activated carbon. With good chemical stability of the modified fibres, and the possibility of easy regeneration and reuse for multiple cycles of treatment without any loss of its adsorption capacity, chemically modified cellulose fibres can be considered as a promising material in the field of sorbent based on renewable resources.

Biographical Notes:

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Sabrine Alila, is associate professor at the Department of Chemistry of the University of Sfax (FSS). Her research topic is concerned with surface chemical modification of cellulose fibres in order to enhance their absorption capacity toward dissolved organic pollutants, including pesticides and herbicides.

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18

Polymers and Biopolymers in Pharmaceutical Technology

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Abstract

Polymers and biopolymers have been used in medicine for centuries. These important materials partly have their own therapeutic effect (active substances), and partly ensure the formulation, stability, and applicability of the dosage form (additives). The discovery of controlled drug delivery systems was a major result of research-development in pharmaceutical technology. In these so-called therapeutic systems, polymers ensuring a predetermined rate of membrane or matrix diffusion are used. Mucoadhesion (adhesion to biological surfaces) and the group of stimuli sensitive (environment sensitive) polymers play an important role in controlling the therapeutic effect.

Keywords: Active substances, additives, biodegradable systems, coated systems, dendrimers, environment sensitive systems, matrix diffusion systems, membrane controlled systems, micro, and nanoparticles, mucoadhesion

18.1 Introduction

Macromolecular substances of natural origin, particularly vegetable resins and colloid solutions prepared from plants, were used for the medicinal treatment of diseases even in the oldest times. The number of polymers and biopolymers used in therapeutics has also risen with the widening range of available medicines and with the development of therapy. With the establishment of the scientific foundations of pharmaceutical technology, a certain change in approach could be observed in the first decades of the 20th century. Macromolecules with a curing effect (active substances) and additives, which are necessary for producing the dosage form but do not have a therapeutic effect of their own, are in a special class. A great number of polymers are used in both fields.

The importance of polymers in pharmaceutical technology is proved by the fact that an independent monograph written by *Czetsch-Lindenwald* [1] was published as long ago as 1963, summarizing the great choice of polymers used in pharmacy. Textbooks and manuals published in the past decades also discuss polymers [2–9] and focus considerable attention on polymeric and biopolymeric

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (525–558)

active substances and additives. Attention must be drawn to the book written by *Müller* [10], which provides a detailed summary of the results of research on colloid drug delivery systems. Excellent monographs [11, 12] give guidance on the abundant choice of additives, including polymer additives.

In the following pages this important group of colloid drug materials will be discussed. Systematization was made along three principles: firstly, the historical development (traditional dosage forms, modern dosage forms), secondly, the purpose of use (active substance, additive, packing material, etc.) and thirdly, the physical-chemical phenomenon which is the basis for the effect of the polymer in the dosage form (e.g. diffusion through the membrane, matrix diffusion, decomposition, etc.).

18.2 Purpose of the Use of Polymers in Pharmacy and Medicine

The fields of pharmaceutical use are illustrated in Figure 18.1. According to the purpose of the application, polymers can be: i) active substances, ii) additives, iii) storing and packing materials, iv) instruments or tools for performing some technological operation (e.g. ion-exchange synthetic resins, polymeric filter materials, etc.), v) tissue-substituting materials, contact lenses and prostheses. Of the items in the fields, we will not be discussing groups iv and v.

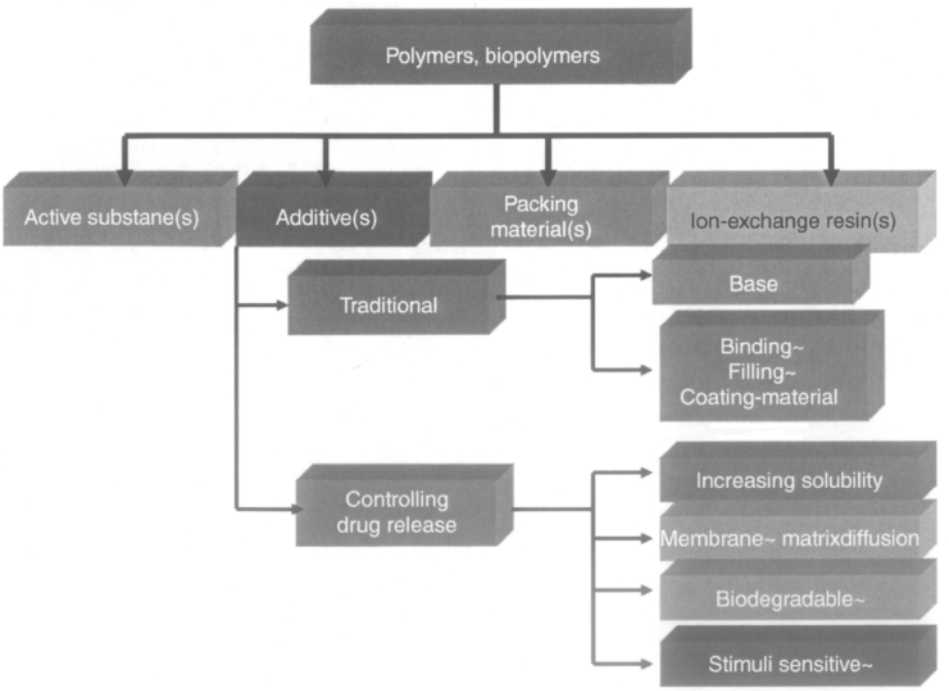

Figure 18.1 Use of polymers in pharmaceutical technology.

The official polymers in the *European Pharmacopoeia 6th Edition* are summarized in Table 18.1.

18.2.1 Active Substances

There are several polymers among the active substances. Classical drugs include disinfectant protein silver (Argentum proteinicum) and colloidal silver (Argentum colloidale), which are mentioned in the pharmacopoeias of several countries. Dextran (its sterile solution) is used for plasma supplementation. Polypeptide and macropeptide hormones (e.g. hormones produced in the hypophysis, pancreas, and ovaries), enzymes (e.g. pepsin, pancreatin), polymer antibiotics (e.g. polymixin, vancomycin) must be mentioned. There are several polymer molecules among cytostatic agents, too [13].

Table 18.1 Polymers in European Pharmacopoeia 6th Edition.

Name, function	Name, function
Acaciae gummi[2,6,7,8,9]	Copolymerisatum methacrylis butylati basicum[3]
Acaciae gumi dispersione desiccatum[2,6,7,8,9]	Dextranum ad iniectabile[11]
Acidum alginicum[2,4,6]	Ethylcellulosum[2,3,6]
Acidum methacrylicum et ethylis acrylas polymerisatum[3]	Gelatina[2,3,5]
Agar[5,6]	Guar galactomannanum[2,4,6,8]
Bentonitum[6,7,9]	Hydroxyethylcellulosum[2,3,5,6,8]
Carbomera[2,5,6]	Hydroxypropylcellulosum[2,3,6,7,8]
Carboxymethylamylum natricum[1,2,4]	Hypromellosum[2,3,5,6,8]
Carmellosum calcicum[4,6,8]	Kaolinum ponderosum[1]
Carmellosum natricum[4,5,6,8]	Macrogola[10]
Carmellosum natricum conexum[4,5,6,8]	Maydis amylum[1,2,4]
Carmellosum natricum subtitutim humile[4,6,8]	Methylcellulosum[5,6,8,9]
Cellulosi acetas[3]	Poly(alcohol vinylicus)[2,3,6,7]
Cellulosi acetas butyras[3]	Polyvidonum[2,3,5,7,8,9]
Cellulosi acetas phthales[3]	Tragacantha[6,8]
Cellulosi pulvis[1,4]	Tritici amylum[1,2,4]
Cellulosum microcrystallinum[1,4]	Xanthani gummi[5,6,8]

[1] filling agent
[2] binding agent
[3] coating agent
[4] desintegrant
[5] gel forming agant
[6] viscosity increasing agent for stabilization
[7] emulsifying agent
[8] wetting agent
[9] suspending agent
[10] ointment and/or suppository base
[11] active agent

18.2.2 Bases for Preparations

The use of polymers in ointment and suppository bases dates back to the middle of the last century. Macrogols (e.g. Macrogol, polyethylene glycol, Carbowax, PEG, PEO) as water-soluble ointment and suppository bases have been used since the 1950s. Plastibase (polyethylene or polypropylene glycol with great molecular weight dissolved in paraffin oil) is a popular ointment base in the USA [1, 2, 4, 7]. Silicones, a specific group of polymers, are used as components in protecting ointments [14, 15].

The aqueous gels of natural macromolecular substances (e.g. alginates, xanthane gum), semi-synthetic cellulose esters (e.g. methyl, hydroxyethyl, hydroxypropyl cellulose, carboxymethyl cellulose sodium), and synthetic polymers (e.g. polyvinyl alcohol, polyvinyl pyrrolidone, polyacrylic acid salts) are used as ointment bases. These systems have numerous advantageous properties: i) it is easy to wash them off the skin, ii) they have good drug release, and iii) their stability is excellent [1, 4, 7]. Aqueous polymer gels are also suitable carriers for rectal drug administration and for the medicinal treatment of the vagina [16–18].

18.2.3 Filling, Binding, Stabilizing and Coating Materials

Tablets represent the dosage form which is the most important and is produced in the highest volume in the pharmaceutical industry. Besides ointments, the formulation of tablets requires the most additives and the use of polymers is possibly the greatest in this field.

Filling materials are used if there is only a small quantity of active substance in the tablet. A classical filling material is starch (e.g. potato, maize, wheat, and rice starch), which also promotes the disintegration of tablets [4, 19]. Recently, cellulose ground to various particle sizes has become increasingly widespread as a binding material (Elcema, Vitacel, Avicel, and Vivapur). These cellulose types are produced from purified cellulose and with their help direct pressing can be performed [20].

In conventional tabletting, powder particles are made to adhere with binding materials (granulation). Generally, the solutions of natural, semi-synthetic, or synthetic polymers are used for granulation (solutions of gelatine, cellulose ethers, and polyvinyl alcohol).

Macromolecular colloid solutions also play an important role in ensuring the stability of disperse systems (e.g. suspensions, emulsions). In the case of emulsions the polymer decreases the rate of separation by increasing viscosity on the one hand, and it has an enthalpy stabilizing effect by adsorption on the surface of the droplets on the other hand [3, 4, 7]. Depending on the concentration of the polymer, a protecting and flocculating effect can be observed during the interaction between suspensions and polymers. If the polymer concentration is low, the polymer adsorbed on the surface of the particles connects the particles into loose floccules. Thereby, the rate of

sedimentation does not decrease but the flocculent sediment can be redispersed well. In higher concentrations the surface of the particles is covered entirely by the polymer. The rate of sedimentation decreases, but the sediment is difficult to shake [2, 3].

The solutions of polymers are also used for coating tablets. Coating may have the following purposes: i) to mask the unpleasant taste of the active substance, ii) to protect the active substance against external effects, iii) to give the product a pleasant look, and iv) to ensure controlled drug release. (The latter is going to be discussed in Chapter 16.4.2.)

Traditional sugar coating has entirely been replaced by film coating. The essence of this operation is that a polymer solution is applied on the core with an appropriate procedure (e.g. spraying, fluidization). After the evaporation of the solvent, the film layer coating the tablet must have appropriate physical properties and give sufficient protection against external effects. The following polymers are used for coating in the pharmaceutical industry: cellulose ethers, cellulose esters, methacrylic acid copolymers, aminoalkyl methacrylate copolymer, methacrylic ester copolymers, polyethylene glycols, polyvinyl pyrrolidone solutions [4, 21].

18.2.4 Polymers Controlling Drug Release

A great and valuable achievement of pharmaceutical technology in the 20th century was the development of controlled-release therapeutic systems.

Therapeutic systems are drug containing preparations or dosage forms from which the active substance(s) is (are) released according to a predetermined program in a specific period of time [22]. This program is usually a physical-chemical phenomenon associated with polymers, such as the swelling of the polymer, the diffusion-controlling effect of the membrane or matrix formed from the polymer, the erosion of the polymer, or a structure-volumetric change arising as a result of the interior milieu. Polymers are able to accelerate or sustain the effect, or possibly to make it pulsatile.

The possibilities of controlled drug release are presented in Figure 18.2. The grouping of the therapeutic systems is shown in Table 18.2 [23, 24].

18.2.4.1 Polymer Solvents, Micelle-forming Agents and Dendrimers

The majority of active substances used currently are not very water-soluble. A very important technological task is to enhance water solubility. Three possibilities concerning polymers are going to be presented: i) formulation of polymer dispersions and polymer solutions, ii) incorporation of apolar active substances into the micelles formed from copolymers, and iii) encapsulation of the active substance in dendrimers or its binding to the functional groups of the dendrimer [7, 9].

It was Sekiguchi and Obi [25] who developed solid solutions. The active substance which is insoluble or poorly soluble in water substitutes the solvent

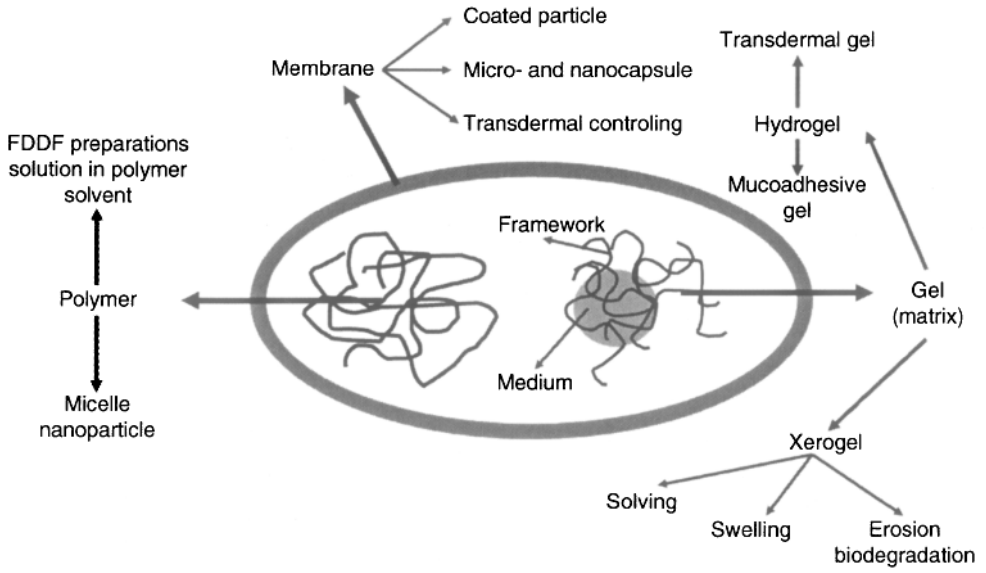

Figure 18.2 Controlling drug liberation with polymers.

Table 18.2 Classification of therapeutic systems with controlled drug delivery [4].

Place of application	• vascular system (infusion) • subcutis (implantation • skin (transdermal) • ocular systems • uterin systems	
Principle of function	* controlling by diffusion	* monolytic systems * reservoir systems
	* controlling by solution	* diffusion * osmosis
	* controlling by chemical way	* erosion of polymers
Source of energy	* physico-chemical energy * chemical energy * mechanical energy * electric or nuclear energy	* diffusion, * osmosis * (bio) degradation * elasticity
Structure of the system	* disperse systems	mcrocapsules liposomes polymersomes micro- and nanospheres
	* coherent systems	gels matrices membranes

molecules in the solid solution or becomes interstitial (Figure 18.3) [26]. Several polymers can dissolve apolar active substance molecules, e.g. dextran, alginates, polyvinyl alcohol, polyvinyl pyrrolidone. These hydrophilic polymers have good swelling properties and form a colloid solution in an aqueous medium, and the active substance with a molecular distribution is released from these solid solutions very fast. That is why they are called FDDF preparations (fast dissolving drug formulations) [6].

Polymeric micelles are called the new generation of colloid drug delivery systems [28]. In an aqueous medium, block and random copolymers associate into micelles with a structure similar to that of surfactants. Active substances which are insoluble or poorly soluble in water can be built into the lipophilic part of the micelle in two ways: i) with dialysis and ii) with the emulsion method (see Figure 18.4).

Dendrimers are regarded as promising drug delivery systems [29-31]. These specifically structured polymers, on the one hand, are able to bind the active substances physically like an inclusion, in the cavities between the branches of the dendrimer, and on the other hand, the dendrimer forms a conjugate with the active groups of the active substance (Figure 18.5).

18.2.4.2 Membrane Diffusion Systems

In membrane diffusion systems the polymer membrane with a given pore size or pore size distribution controls the diffusion of the active substance from the drug reservoir. Dosage forms with membrane-controlled drug delivery can be coated tablets, coated granules or pellets, or so-called multiparticulate systems on which various coats are applied. One possibility for transdermal drug administration is the transdermal patch controlled with a membrane [4–7, 34–39].

18.2.4.2.1 Coated Tablets, Granules, Pellets

In the case of granules, pellets and tablets the polymer film coat not only gives protection but it also makes controlled drug release possible (Table 18.3).

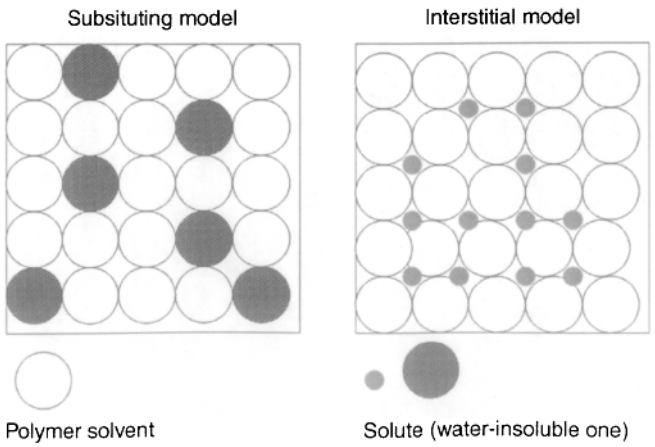

Figure 18.3 Types of solid solutions.

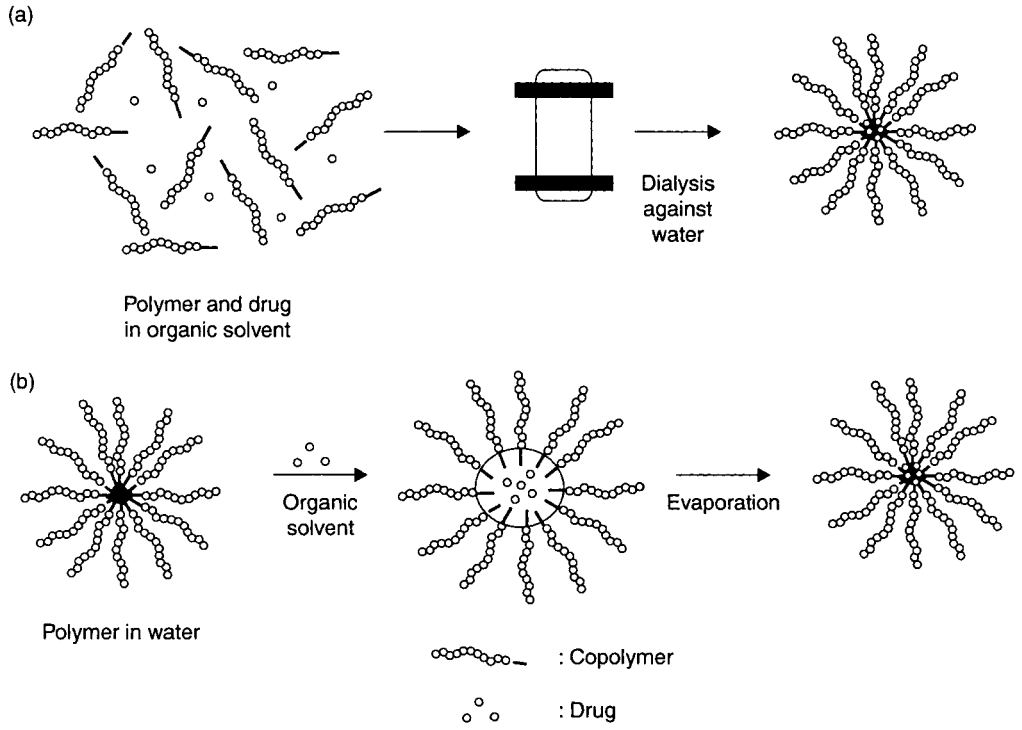

Figure 18.4 Polymer micelles. Drug loading of polymer micelles by (a) dialysis and (b) oil-in-water emulsion method [27].

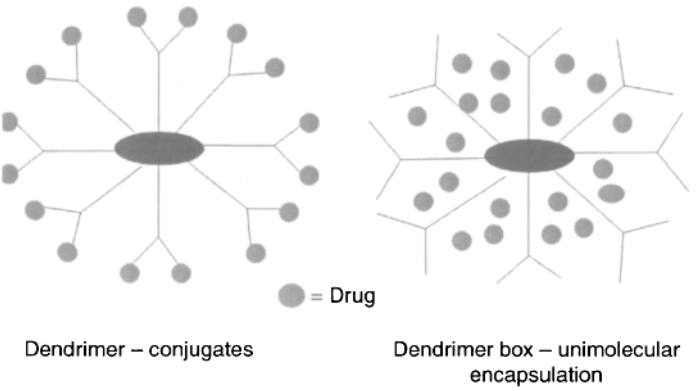

Figure 18.5 Application of dendrimers as solvent of water insoluble substances [30].

Solid state drug release systems controlled with coating are called membrane diffusion or reservoir systems [36, 37]. The process of drug release is illustrated in Figure 18.6. The driving force of the process is diffusion and the phenomenon of osmosis [22, 37, 38, 40].

The process is a complex phenomenon consisting of several elements. The following sub-processes must be mentioned: i) wetting of the system with water and digestive juices, ii) penetration of water into the product through

Table 18.3 Some commonly used film-coating materials [8].

Typ	Full name (Abbreviation)	Soluble in
Nonenteric	Methylcellulose (MC)	Cold water, GI fluids, organic solvents
	Ethylcellulose (EC)	Ethanol, other organic solvents
	Hydroxyethylcellulose (HEC)	Water and GI fluids
	Methylhydroxyethylcellulose (MHEC)	GI fluids
	Hydroxypropylcellulose (HPC)	Cold water, GI fluids, polar organics; such as anhydrous lower alcohols
	Hydroxypropylmethylcellulose (HPMC)	Cold water, GI fluids ; methanol/methylene chloride, alcohol/fluorhydrocarbons
	Sodium carboxymethylcellulose (Na-CMC)	Water and polar organic solvents
	Povidone (PVP)	Water, GI fluids, and IPA
	Polyethylene (PEGs)	Water, GI fluids, some organic solvents
Enteric	Shellac	Aqueous, if pH 7
	Cellulose acetate (CAP)	Acetone, ethyl acetate/IPA
	Polyvinyl acetate phthalate (PVAP)	As above, if pH > 5.0
	Hydroxypropylmethylcellulose phthalate (HPMCP)	As above, if pH > 4.5
	Polymers of methacrylic acids and its esters	Eudragit L pH > 6 Eudragit S pH > 7

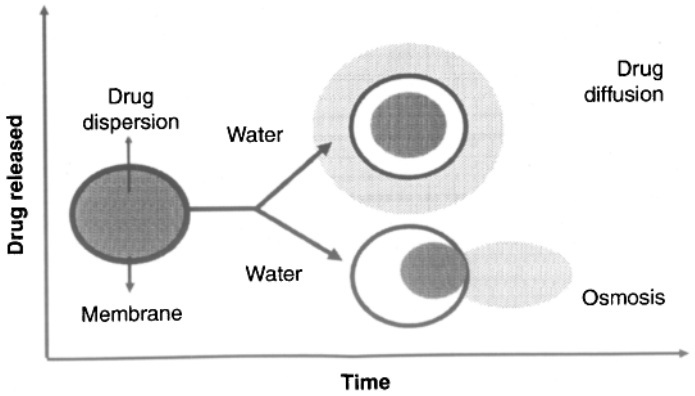

Figure 18.6 Schematic drawing illustrating drug release from membrane controlled drug delivery systems (membrane diffusion systems and osmeotic systems).

the pores of the coat or the continuous polymer film, iii) dissolution of the drug (and the additives), iv) formation of water-filled pores, v) closing of the pores as a result of the swelling of the polymer, vi) development of hydrostatic pressure inside the closed system, vii) flowing out of the active substance caused

by the hydrostatic pressure, viii) appearance of cracks in the membrane controlling diffusion, and ix) diffusion of the active substance with the given diffusion coefficient [39].

There have been several proposals for the mathematical description of the process [37, 39, 42, 43]. Linhardt [37] and Siepmann [39] proposed the following linear relationship to describe the kinetics of the process:

$$M_t = D\frac{AC_s t}{h} \tag{18.1}$$

where M_t is the active substance released in time t, D is the diffusion coefficient, the surface of the membrane, C_s is the dissolution of the active substance in the membrane, h is the thickness of the membrane.

Baker [44] proved the validity of a more complex relationship:

$$M_t = \frac{ADKc_t}{h} = \frac{ADK}{h}\frac{c_0 - c_x}{V} \tag{18.2}$$

where c_t is the constant describing the concentration change of the active substance, c_0 is the initial quantity of the active substance in the therapeutic system, c_x is the active substance after time t, K is the partition coefficient between the membrane and the reservoir, and V is the volume of the reservoir.

18.2.4.2.2 Multiparticulate Systems

The essence of multiparticulate systems is that several drug delivery systems controlled in various ways are present in one preparation [37, 41, 45, 46]. The two basic types of the preparations are shown in Figures 18.7 and 18.8.

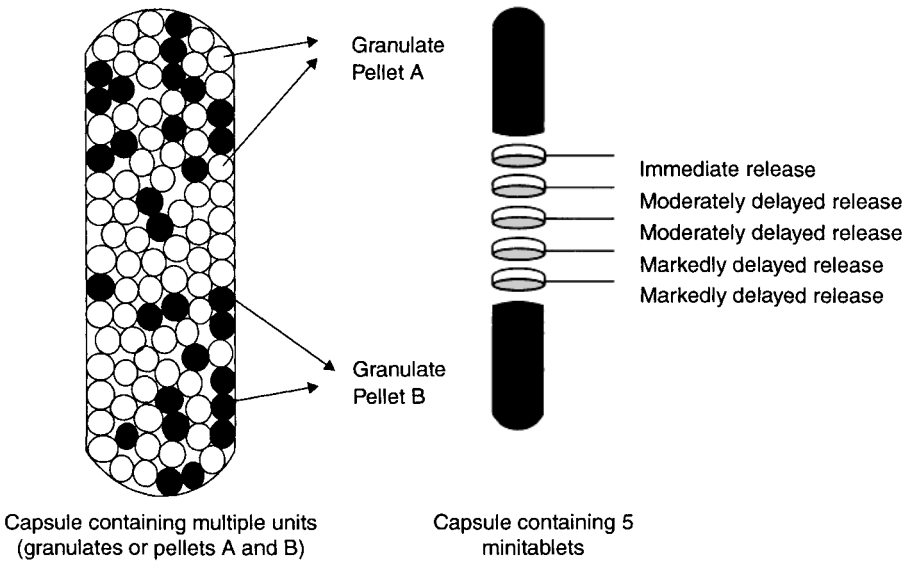

Figure 18.7 Multiparticulate drug delivery systems [36].

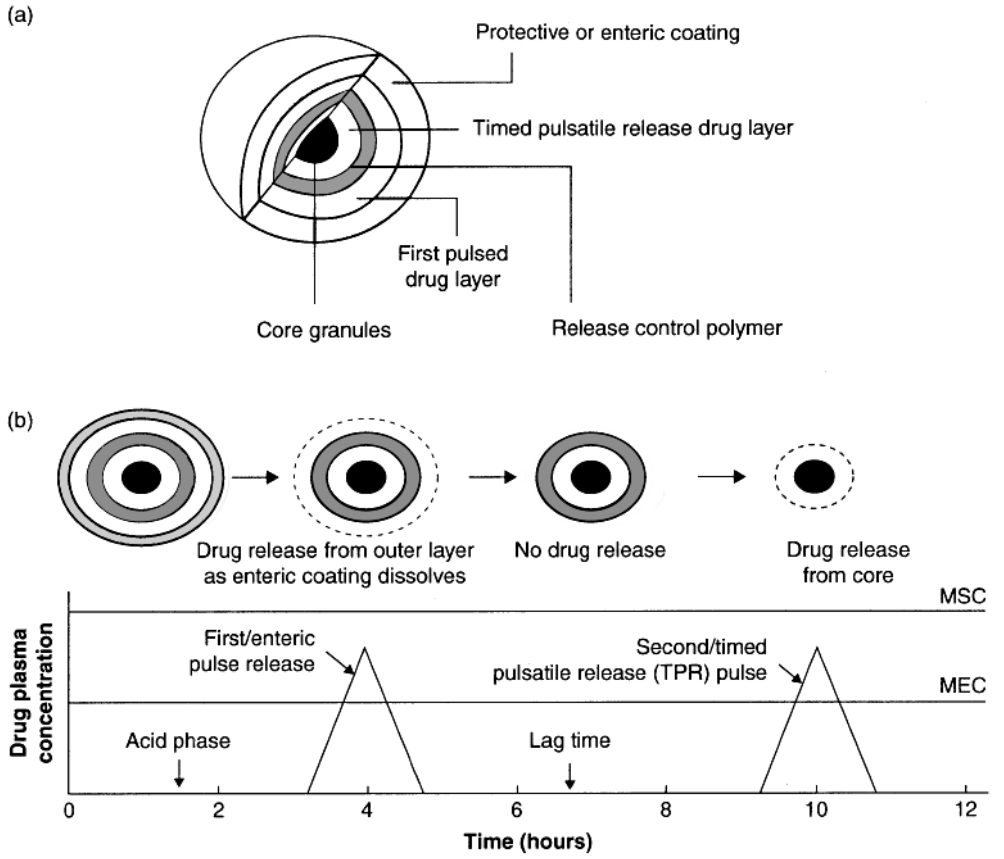

Figure 18.8 Hypothetical design and plasma drug profile of multiple pulsatile system. (A) Design of a pellet with multiple coatings and (B) predicted bi-modal plasma concentration profile [41].

18.2.4.2.3 Micro- and Nanoparticles

Micro- and nanoparticles are very fine particles with a small linear size. The size range of microsystems is 100–1000 μm, while nanoparticles fall within the lower range of the colloid dimension, their typical size is between 15–300 nm [5–7, 57, 58]. The pharmaceutical application of these important systems, the process of controlled drug delivery, is dealt with in several publications. Some of the major, comprehensive publications are cited [47–56].

Figure 18.9 shows the schematic picture of the structured particles and the coated micro- and nanoparticles as well as drug liberation from them.

The various types of nanoparticles and their major properties are presented in Figure 18.10.

There are various methods of production. The most important ones are: i) nanoprecipitation with the evaporation of the solvent, ii) emulsification-diffusion, iii) emulsification-coacervation, iv) coating with polymer, and v) layer by layer method [55, 57] (Figure 18.11).

The coat formed around the active substance particles or droplets is mostly biodegradable. In addition to polymers of natural origin, poly-ε-caprolactone

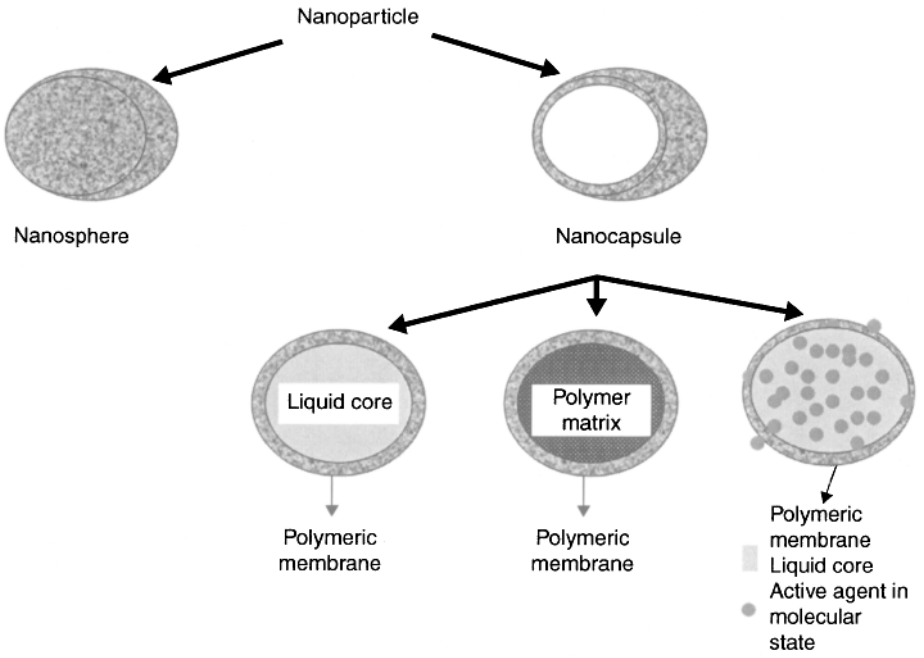

Figure 18.9 Nanoparticles, nanospheres, nanocapsules.

(PCL), polyhydroxybutyrate-co-hydoxyvalerate copolymer (PHBHV), and polylactic acid derivatives (PLA) are generally used as wall-forming agents [6, 47, 49, 55, 57]. Some nanocapsulated active substances must also be mentioned: indomethacin, progesterone, estradiol, eugenol, hinokitol, and 4-nitroanisol [55].

18.2.4.2.4 Membrane-controlled Transdermal Drug Administration

Drug administration through the intact skin, transdermal therapy, was realized at the end of the 20th century with the development of transdermal therapeutic systems [22, 59]. Various polymers are necessary for this.

One of these types is the membrane-controlled transdermal therapeutic system, which is outlined in Figure 18.12. These systems consist of the following parts: i) covering membrane, ii) drug reservoir, iii) micropore membrane controlling drug delivery, and iv) adhesive contact surface. (Further types of transdermal systems are going to be described in Chapter 16.2.4.3.3). The most commonly used membranes are polyethylene vinyl acetate and polyethylene [60–62].

The rate of liberation, diffusion through the membrane, can be described with the help of the following equation [59]:

$$\frac{dM}{dt} = \frac{C_R}{\frac{1}{P_m} + \frac{1}{P_a}} \tag{18.3}$$

where M is the quantity of the active substance liberated in time t, C_R is the concentration of the active substance in the reservoir, P_a and P_m is the adhesive

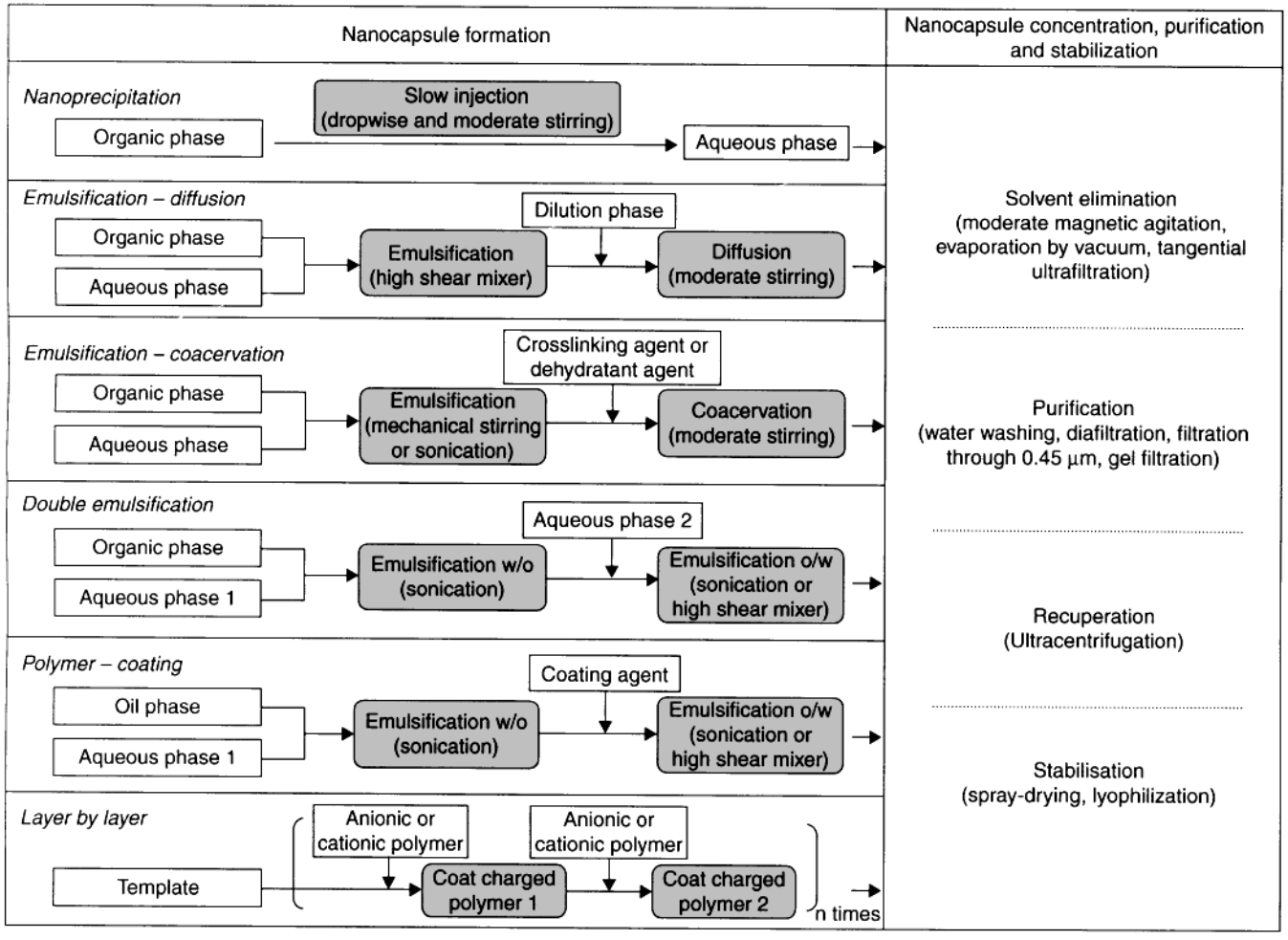

Figure 18.10 Nanoparticulate drug delivery systems formed by amphiphilic block co-polymers and their characteristics [55] .

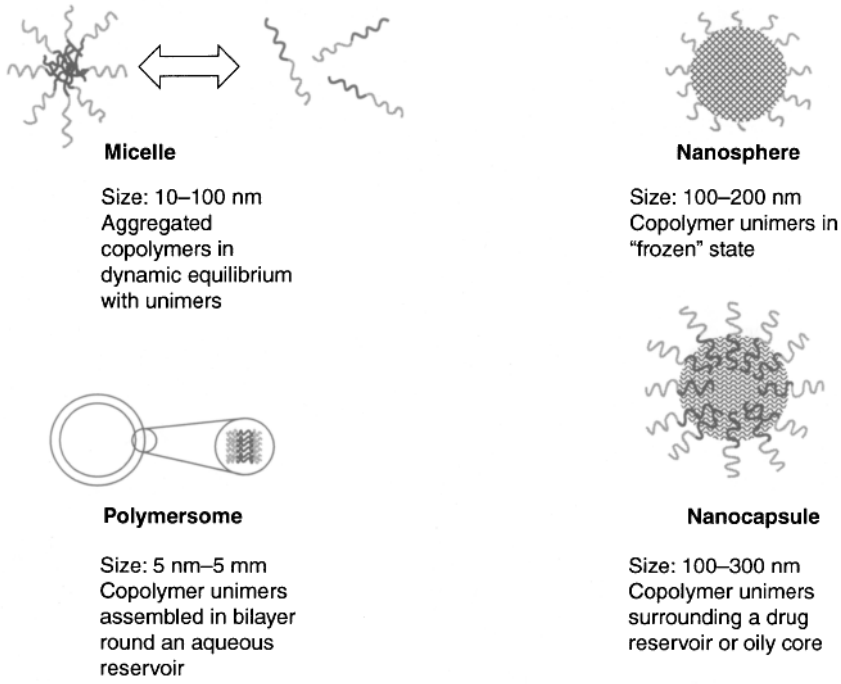

Figure 18.11 General procedure of different methods for the preparation of nano-capsules [52].

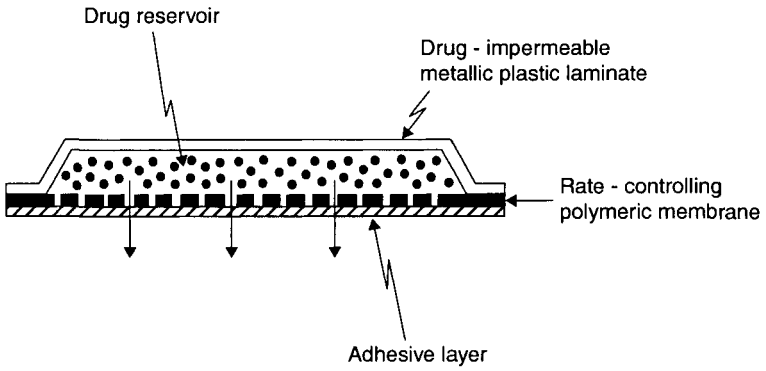

Figure 18.12 The cross-section view of a membrane controlled transdermal drug delivery system, showing various major structural components [59].

layer and the membrane permeability coefficient, respectively. Considering the permeability of the membrane and the adhesive layer:

$$P_m = \frac{K_{m/r} D_m}{h_m} \tag{18.4}$$

$$P_a = \frac{K_{a/m} D_a}{h_a} \tag{18.5}$$

where K is the partition coefficient, D is the diffusion coefficient, and indices m, r and a denote the membrane, the reservoir and the adhesive layer, respectively. With the substitution of equations 16.4 and 16.5 into equation 16.3, the quantitative relationship of the factors determining the diffusion of the active substance is given.

$$\frac{dM}{dt} = \frac{K_{m/r}K_{a/m}D_aC_R}{K_{m/r}D_mh_a + K_{a/m}D_ah_m} \tag{18.6}$$

18.2.4.3 Matrix Diffusion Systems

In matrix diffusion systems the diffusion of the active substance is determined by the structure of the polymer matrix and its changes arising in an aqueous medium. The properties of the matrix which must be mentioned include the porosity of the matrix, the tortuosity of the pores, and the dissolution of the active substance in the matrix. Swelling and erosion in the biological milieu are also important factors in controlling drug release. Matrix diffusion systems are also called monolithic systems [9, 36, 37, 38, 40]. The scheme of the process of drug release is presented in Figure 18.13.

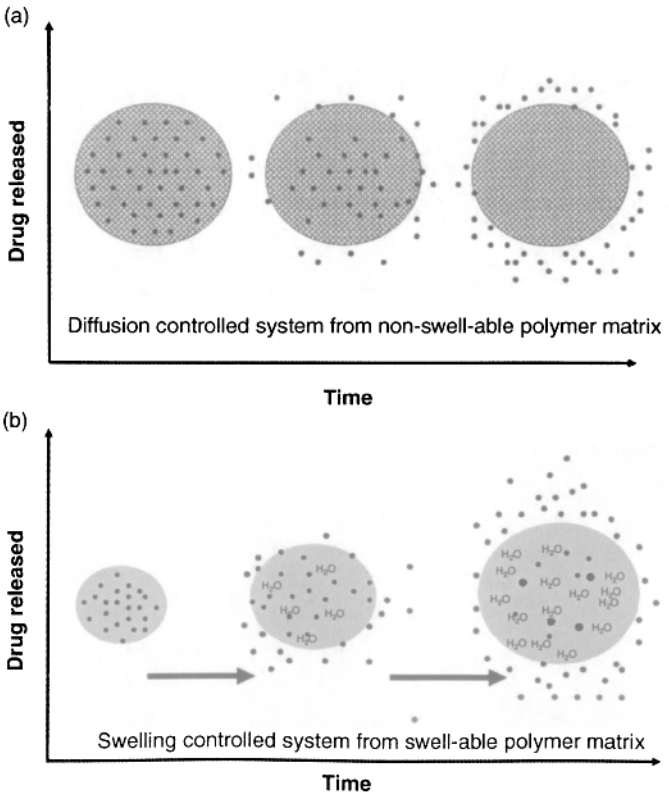

Figure 18.13 Diffusion controlled drug delivery systems, (A) Matrix is unswelling device, (B) Matrix is a swelling controlled one.

Several conceptions were proposed for the mathematical description of the process [63–66].

According to the general model of Higuchi [64], the quantity of the active substance released (M_t) is determined by the surface (A), the diffusion coefficient (D), the initial concentration of the active substance (C_0), and the solubility of the active substance (C_s):

$$M_t = A\sqrt{D(2C_0 - C_s)C_s t} \qquad \text{where } C_0 > C_s \tag{18.7}$$

Higuchi [64] took into consideration the structure of the matrix, and also the interaction between the matrix and the active substance. In the case of a hydrophobic matrix and an active substance insoluble in the matrix, the following equation applies:

$$M_t = \sqrt{D\frac{\varepsilon}{\tau}(2C_0 - \varepsilon C_s)C_s t} \tag{18.8}$$

where ε is the volume of the matrix pores and τ is the tortuosity of the pores.

If the active substance dissolves in the hydrophobic matrix or the matrix is saturated with the solution of the active substance, the following equation is obtained:

$$M_t = \sqrt{2Co\varepsilon\frac{D}{\tau}t} \tag{18.9}$$

In the case of a hydrophilic matrix and suspended active substance (insoluble in the matrix), the time course of liberation can be described with the following equation:

$$M_t = \sqrt{D\varepsilon(2\frac{C_s}{V} - \varepsilon C_s)C_s t} \tag{18.10}$$

where V is the volume of the hydrated matrix.

If the active substance dissolves in the hydrophilic matrix or water, the following equation will result:

$$M_t = \sqrt{2\varepsilon\frac{C_0}{V}\frac{D}{\tau}t} \tag{18.11}$$

The equation proposed by Rittger and Peppas [65] is the following:

$$\frac{M_t}{M_\infty} = Kt^n \tag{18.12}$$

where M_∞ is the quantity of the active substance released in an infinite period of time, K is constant, n is the constant characteristic of the kinetics of the process.

If the value of n is between 0.43 and 0.5, diffusion according to Fick's law takes place, if it is higher than that, diffusion does not follow Fick's law.

The equation by Peppas and Sahlin connecting diffusion according to Fick's law and not according to Fick's law is the following:

$$\frac{M_t}{M_\infty} = k_1 t^n + k_2 t^{2n} \tag{18.13}$$

where k_1 is the constant of diffusion according to Fick's law, k_2 is the constant of diffusion not according to Fick's law.

The general formula of the liberation equation is [37]:

$$\frac{M_t}{M_\infty} = kt^n \tag{18.14}$$

18.2.4.3.1 Matrix Tablets and Implants

Matrix tablets appeared in the 1980s and became popular very fast as they had several advantageous properties. Their advantages include: i) the active substance can be dispersed in the matrix homogeneously, ii) they can be produced more easily than reservoir systems (membrane-coated systems, see Chapter 16.2.4.2), and iii) they also can be used with active substances with a high molecular mass [6, 8-10, 36, 37]. The most frequently used matrix-forming materials ensuring controlled drug release are summarized in Table 18.4.

In respect to their structure, implants are similar to matrix tablets. They are produced with pressing or extrusion. Currently they are used for hormone replacement resulting from the insufficient functioning of the endocrine system [70–72]. Polyethylene glycol and chitosan are the most frequently used in implants [71, 72].

18.2.4.3.2 Microspheres, Nanospheres

The use of compact micro- and nanospheres as drug delivery systems offers several benefits. The advantages of micro- and nanospheres are: i) small particle size, and consequently a large specific surface, ii) they can be suspended easily and suspensions containing nanoparticles do not sediment, iii) they have good adhesion to the organs and tissues in the living organism, iv) they penetrate into the cells and thus take part in subcellular processes directly, v) they can be sterilized with membrane filtration, vi) they can be used for controlled drug release, and vi) the active substance in this form can be targeted directly [47, 48, 49, 73, 74].

As to their structure, micro- and nanospheres can be of two types: i) micelles formed from copolymers and ii) porous spheres in micrometer size or in the colloid size range.

18.2.4.3.3 Transdermal Drug Administration Controlled with Matrix Diffusion

Drug administration through the skin was first achieved with hydrogel-type patches, that are polymer matrixes [4, 22, 61, 75]. Matrix diffusion is characteristic

Table 18.4 Some commonly used controlled release polymers or excipients forming matrix-systems [8].

Type	Polymers or other additive
Hydrophilic ~	Acrylic acid Acrylic acid derivates/ esters Carboxymethyl cellulose (CMC) Ethylcellulose (EC) Hydroxypropylcellulose (HPC) Hydroxypropylmethylcellulose (HPMC) Methylcellulose (MC) Poly(acrylic acid) (PAA) Poly(aminobutyl glycolic acid) (PAGA Poly(caprolactone) (PCL) Poly(Lactic acid) (PLA) Poly(lactic co-glycolic acid) (PLGA) Poly(vinyl acetate) (PVAc) Poly(vinyl alcohol) (PVA) Poly(vinyl pyrrolidone) (Povidone, PVP) Polyethylene glycol (PEG)
Hydrophobic~	Carnauba wax Glyceryl monostearate Glyceryl palmitostearate Hydrogenated vegetable oil Paraffin White wax
pH dependent ~	Cellulose acetate phthalate (CAP) Hydroxypropylmethyl cellulose (HPMCP) Poly(methacrylates) Poly(vinyl acetate phthalate) (PVAP) Shellac Zein
Surface active ~	Pluronics

of three transdermal systems with different structures: i) matrix dispersion system (Figure 18.14), ii) system controlled with adhesive layer, (Figure 18.15) and iii) the so-called microreservoir-type system (Figure 18.16) [22, 59].

In a matrix dispersion system the active substance is in the polymer matrix reservoir in a dispersed (dissolved or suspended) state and diffusion is controlled by the structure of the matrix. The rate of release is described by a relationship similar to (16.7) Higuschi's equation [59]:

$$\frac{dM}{dt} = \sqrt{\frac{C_0 C_s D}{2t}} \tag{18.15}$$

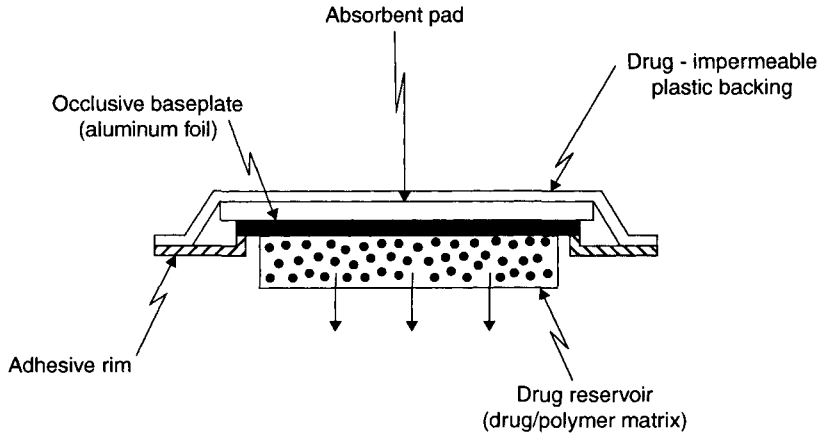

Figure 18.14 The cross-section view of a matrix dispersion transdermal drug delivery system, showing various major structural components [59].

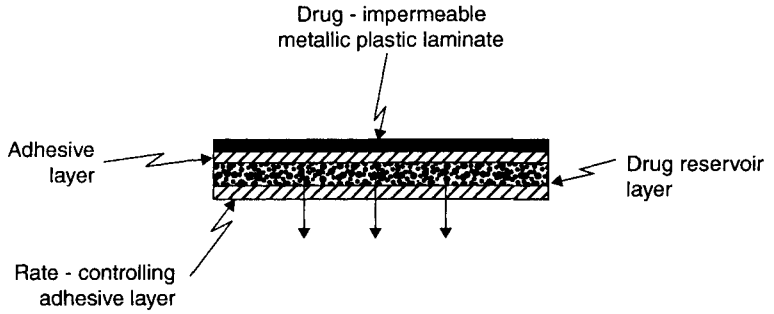

Figure 18.15 The cross-section view of adhesion diffusion controlled transdermal drug delivery system, showing various major structural components [59].

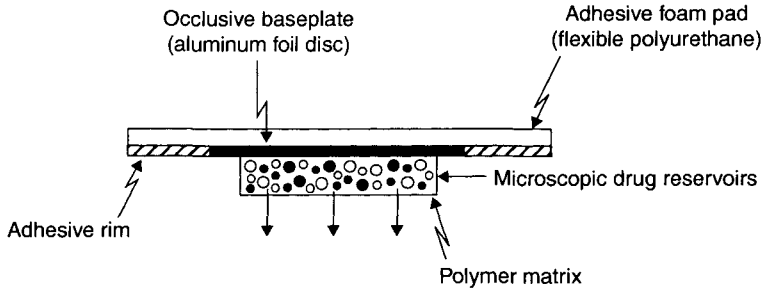

Figure 18.16 The cross-section view of a microreservoir-type transdermal drug delivery system, showing various major structural components [59].

where C_0 is the initial concentration of the active substance, C_s is the solubility of the active substance in the matrix, and D is the diffusion coefficient. Constants describing the morphology of the matrix (porosity, tortuosity, or the pores) are absent from the equation.

The system controlled with an adhesive layer consists of two polymer gels with different structures. The rate of diffusion is given by the flow of the active substance in the adhesive layer [59]:

$$\frac{dM}{dt} = \sqrt{\frac{K_{a/r} D_a C_s}{h_a}} \tag{18.16}$$

where K is the partition coefficient between the adhesive layer (a) and the reservoir (r), D_a is the diffusion constant of the active substance in the adhesive layer, C_r is the concentration of the active substance in the reservoir, and h_a is the thickness of the adhesive layer.

Microreservoir-type transdermal patches consist of two reservoirs, a polymeric matrix and liquid droplets of microscopic size containing the active substance. The reservoir is surrounded with a polymer membrane [59]. Thus multi-step diffusion takes place during the release of the active substance: from the matrix into the acceptor medium (skin), from the liquid droplet into the membrane, from the membrane into the matrix. Consequently, the mathematical relationship describing this phenomenon is more complicated than the previous ones:

$$\frac{dM}{dt} = \frac{D_p D_s a K_p}{D_p h_d + D_s h_p a K_p} \left[\beta S p - \frac{D_l S_l (1-\beta)}{hl} \left(\frac{1}{K_l} + \frac{1}{K_m} \right) \right] \tag{18.17}$$

In the equation, α is the proportion of the active substance dissolved and suspended in the gel phase, β is the proportion between the quantities of the active substance found in the droplets and dissolved in the polymer membrane, K is the partition coefficient. Index l shows the partitioning between the liquid and the matrix, index m is the partitioning between the membrane bordering the liquid droplets and the matrix, and index p is the partitioning between the membrane and the acceptor medium. (The same applies to indexes l, m, and p of diffusion constant D.)

Active substances used in membrane diffusion transdermal systems can be the following: steroid hormones, clonidine, scopolamine, nicotine, nitroglycerine, dexamethasone, propranolol, and analgesics (e.g. phentanyl, buprenorphine), [60, 61, 75].

18.2.4.4 Biodegradable Systems

One of the interesting components of the drug release controlling effect of drug delivery polymer matrixes is the degradation of the polymer matrix in the biological medium, in consequence of which the active substance is released [37, 38, 77–80] (Figures 18.17 and 18.18).

Bioerosion and biodegradation are differentiated in literature. Degradation means the splitting of the polymer chains, in the course of which oligomers and monomers are formed. On the other hand, erosion is the material loss arising in the mass of the polymer [77].

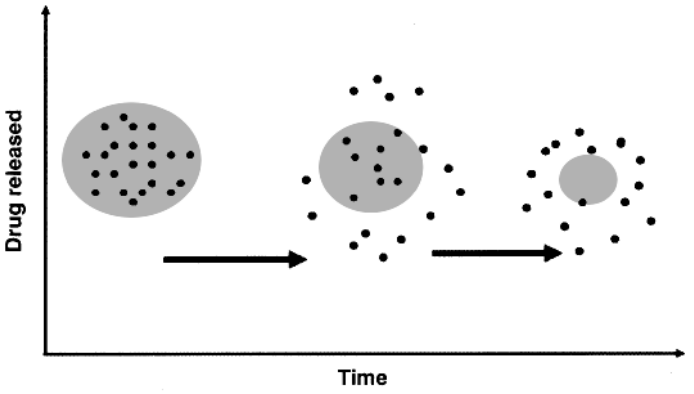

Figure 18.17 Erosion controlled drug release from polymer matrix.

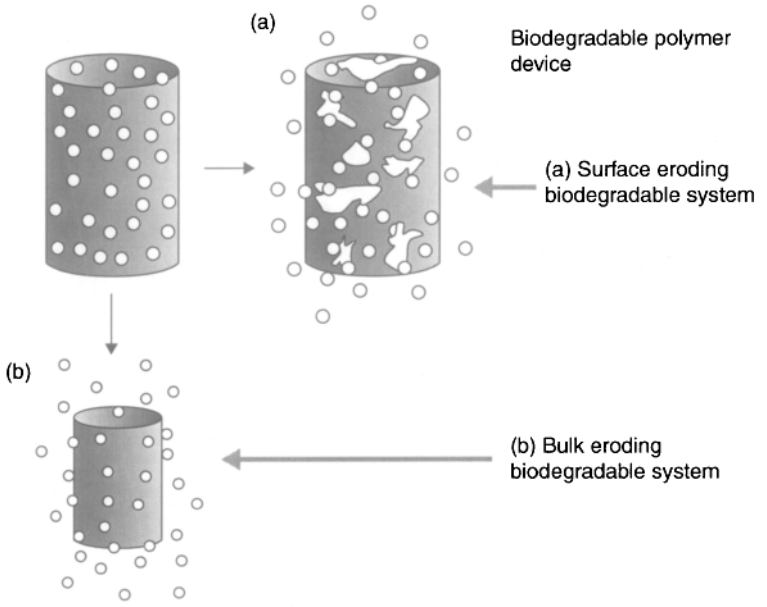

Figure 18.18 Drug delivery from biodegradable systems, (A) surface eroding biodegradable system, (B) bulk eroding system [38].

The erosion of the polymer matrix depends on several factors (e.g. composition, attachment of the active substance to the matrix, morphology, and geometry of the matrix), and these determine the kinetics of drug release. This is the result of several consecutive and concomitant physical and chemical processes. Sub-processes are the following: i) penetration of water into the matrix, ii) dissolution of the active substance, iii) degradation of the polymer, iv) formation of pores, v) diffusion of the active substance and the degraded polymer within the matrix, vi) recrystallization of the degradation products (mono- or oligomers) within the matrix, vii) microenvironmental pH change in the

pores of the polymer matrix due to the effect of the decomposition products, viii) diffusion of the active substance and the polymer decomposition products outside the pores, ix) diffusion of hydrogen and hydroxyl ions from the biological environment into the matrix, x) autocatalytic processes due to the effect of the decomposition products, xi) osmotic effects, xii) swelling of the polymer, xiii) flow processes, and xiv) adsorption/desorption processes [77].

There are two types of biodegration: biodeterioration, which is surface degradation and biofragmentation, which is a lytic process requiring energy. Biodeterioration is the result of a physical, chemical or enzymatic process. Biofragmentation, the splitting of the polymer molecule in the living organism, is necessary because the polymer, as a result of its great molecular mass, cannot penetrate through the cell wall or the membrane of the cytoplasm. The energy needed for splitting can be light, mechanic, chemical, or biological. Biofragmentation takes place as a result of enzymatic hydrolysis, enzymatic oxidation and radical oxidation [78]. Erodable or biodegradable polymeric carriers are chosen mainly for the formulation of oral and implant preparations.

18.2.4.5 Environment Sensitive Systems

To be environment sensitive or stimuli sensitive means that the polymer responds to relatively small changes in the environment with substantial physical or chemical changes. These polymers are also called 'intelligent' polymers [7, 9].

The change of the polymer can be: i) reversible precipitation or gelation, ii) reversible adsorption on some surface, iii) reversible collapse of graft polymer, and iv) reversible collapse of hydrogel [7] (Figure 18.19).

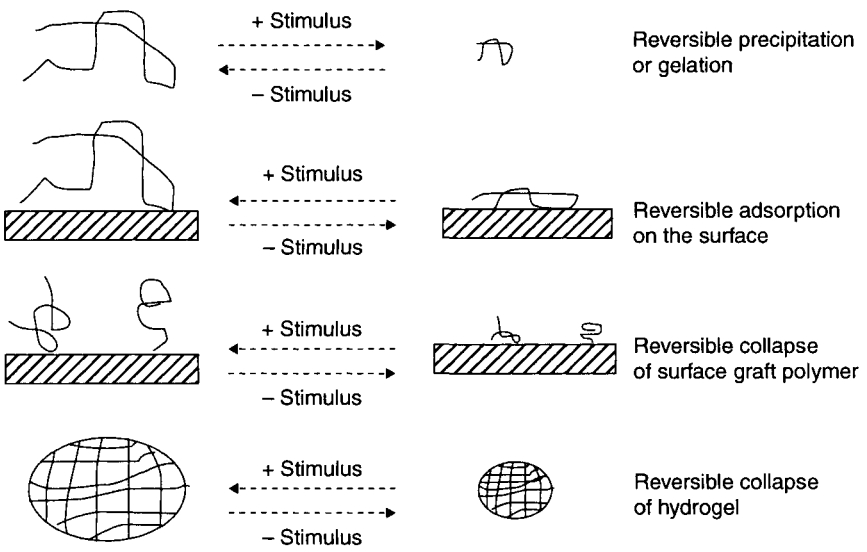

Figure 18.19 Schematic representation of stimuli sensitive polymers in solution or in surface and as hydrogel [7].

The most frequent change of drug delivery systems formulated from stimuli sensitive polymers is swelling – collapse [81] (Figure 18.19). Triggering factors are: i) temperature, ii) pH, iii) light, iv) ultrasound, v) electric or magnetic field of force, vi) pressure, vii) various chemical substances (metals, acids, carbamide), viii) various biochemical materials (glucose, antigens, thrombin), and ix) pathophysiological phenomena (inflammation) [81–83]. These factors are summarized in Figure 18.20.

Some drug release systems based on stimuli sensitivity are shown in Table 18.5.

Thermoresponsive polymer gels are the most thoroughly studied drug release systems, in which the on-off phenomenon is induced by the change of temperature [82–88]. Not only hydrogels react to a pH change, but also liposomes and niosomes consisting of surfactants and polymers with complicated structure [89]. Hydrogels which control the flowing out of the active substance with biochemical reactions produced by glucose and proteins (e.g. enzymes, antigens) have a special place among environment sensitive drug release systems [90]. Pulsatile drug release can also be achieved with intelligent polymer drug delivery systems [91, 92], and the active substance can also be targeted (binding only to tumour cells) [93].

18.3 Administration of Active Substances through the Mucosa of Body Cavities with the Help of Polymers and Biopolymers

In addition to the traditional ways of administration (e.g. oral administration, injections into the muscle or vein), the administration of the drug through the mucosa of body cavities is becoming increasingly common [7, 8, 10, 19, 29]. Administration through the mucosa has several advantages: i) it is non-invasive,

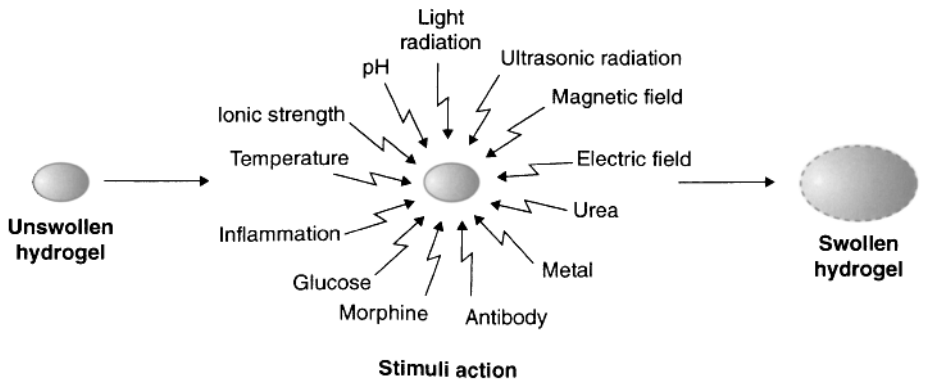

Figure 18.20 Stimuli responsive swelling of hydrogels [81].

Table 18.5 Stimuli-responsive hydrogels in drug delivery [81].

Stimuli	Polymer	Drug (active agent)
Magnetic filed	Ethylene –co-vinylacetate (EVAc)	Insulin
Untrasonic radiation	Ethylene –co-vinylacetate (EVAc)	Zinc bovine insulin
	Ethylene-co-vinylalcohol	Insulin
Electric field	Poly(2-hydroxyethyl methacrylate (PHEMA)	Propranolol hydrochloride
Glucose	Ethylene –co-vinylacetate (EVAc)	Insulin
Urea	Methyl vinyl ether-co-maleic anhydride	Hydrocortisone
pH	Chitosan-poly(ethylene oxide) PEO	Amoxicillin, metronidazole
	Poly(acrylic acid)-PEO	Salicylamide, Nicotinamide, Clonidine hydrochloride, Prednisolone
	Gelatin-PEO	Riboflavin
	PHEMA	Salicylic acid
	Poly(acrylamide-co-maleic acid)	Terbinafine hydrochloride
Temperature	*N*-vinyl pyrrolidone,Polyethylene glycol diacrylate, Chitosan	Theophylline 5-fluorouracil
pH and temperature	Poly(N-isopropyl acrylamide)	Heparin
	Poly(N-isopropyl acrylamide-co. Butyl methacrylate-co-acrylic acid)	Calcitonin

it does not cause pain to the patient, ii) the active substance is protected from the damaging effects of the gastrointestinal tract (e.g. hydrochloric acid, enzymes), iii) the active substance bypasses the metabolizing effect of the liver (first pass effect), and iv) there is good contact between the drug release system (polymer) and the mucosa.

18.3.1 Mucoadhesion

Good mucoadhesion is indispensable to drug administration through the mucosa. Mucoadhesion is a form of bioadhesion. Bioadhesion is when, from among the two components of adhesion (surface and substrate), one or both belongs/belong to the living organism [7, 94]. Dermoadhesion is when the biological surface is the skin and mucoadhesion when it is the mucosa.

The phenomenon is explained on a tissue and cell level [94–98]. The qualitative description of the phenomenon is the following: The mucosa of the body cavity enters into contact with the polymer carrying the drug. First the two aqueous systems moisten each other, then the polymer and the mucin of the

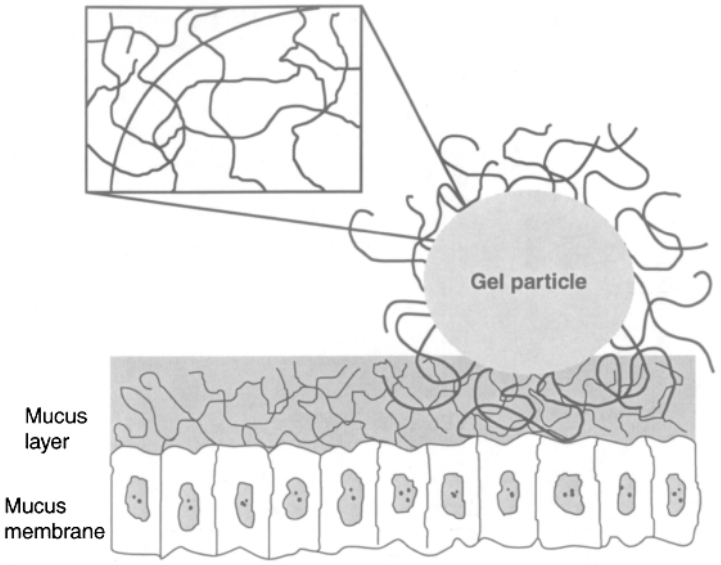

Figure 18.21 Schematic diagram representing the interpenetration between tethered chains and mucus gel layer [97].

mucosa penetrate into each other (interpenetration), as a consequence of which physical and physical-chemical interactions develop between the two polymers (bonds of secondary valence type) [95–97]. This is illustrated in Figure 18.21.

Four theories have been proposed for the theoretical explanation of this phenomenon. i) The two polymers have an electric double layer, between which electron transfer takes place. ii) Mechanical connection, one polymer fits into the other like a key into the lock. iii) According to the adsorption theory, the two polymers enter into an adsorption relationship with each other. iv) Interaction between the participants results from wetting [96].

The phenomenon is also explained on a cellular level [98]. According to this explanation, the drug carrier can bind to the surface of the cell, it enters the cell in the form of a lysosome and transport takes place through the cytoplasm.

Several methods have been developed for the experimental investigation of the process and for the quantitative description of mucoadhesion. These methods can be classified into the following groups: i) methods based on measuring viscosity [99-102], ii) separation of the substrate and the adhesive with microscales based on horizontal or vertical force measurement [94, 95, 103-105], the most up-to-date means of which is the so-called texture analyser [106], iii) *in vitro* or *in vivo* cell culture examinations [107-109], and iv) animal experiments [94, 105, 110].

The bioadhesion of a few polymers is compared in Table 18.6 [94].

18.3.2 Mucoadhesive Preparations in the Gastrointestinal Tract

The research on drug administration through the mucosa of the oral cavity dates back to the beginning of the 1980s [111]. Buccal preparations are

Table 18.6 Classification of Bioadhesive Polymers [94].

Test polymer	Mean % adhesive force*	Standard deviation	Qualitative bio(muco) adhesive property
Sodium carboxymethyl cellulose	192.4	12.0	
Poly(acrylic acid)	185.0	10.3	
Tragacantha	154.4	7.5	
Poly(metyl vinylether-co-maleic anhydride)	147.7	9.7	Excellent
Poly(ethylene oxid)	128.6	4.0	
Methylcellulose	128.0	2.4	
Sodium alginate	126.2	12.0	
Hydroypropylmethylcellulose	125.2	16.7	Satisfactory
Karaya gum	125.2	4.8	
Methylethylcellulose	117.4	4.2	
Soluble starch	117.2	3.1	Fair
Gelatin	115.8	5.6	
Pectin	100.0	2.4	
Poly(vinyl pyrrolidone)	97.6	3.9	
Poly(ethylene glycol)	96.0	7.6	
Poly(vinyl alcohol)	94.8	4.4	Poor
Poly(hydroxyethylmethacrylate)	88.4	2.3	
Polyhydroxypropylcellulose	87.1	13.3	

* related to Pectin as 100%

advantageous for several active substances – first of all peptides (e.g. insulin) decomposing in the stomach and topical preparations for the treatment of the diseases of the mucosa must be mentioned [112].

Absorption through the oral mucosa can take place in two ways: i) inter- or paracellular pathway, which is a passive transport through the lipid matrix between the cells and ii) trans- or intracellular pathway through the cell walls, which can be either passive or active transport. The following dosage forms may be used: i) polymer-coated nanoparticles, ii) special tablets, iii) polymer films, and iv) gels (patches) [112–119].

It is also possible to ensure that the mucoadhesive preparation is retained in the stomach until the active substance is dissolved completely [120] or the active substance is released from the preparation only in the colon [121].

18.3.3 Drug Administration through the Nasal Mucosa

The nasal mucosa offers a great possibility for the administration of several active substances. Its advantages are: i) rapid absorption, ii) immediate onset

of effect, iii) first pass metabolism can be avoided, iv) good possibility for chronic treatment, and v) easy application, popular method of administration.

The dosage forms used are: viscous solutions, gels, adhesive nanoparticles, and adhesive powders [123–127].

18.3.4 Mucoadhesive Preparations on the Mucosa of the Eye

The property of polymers to sustain effect was first recognized in ophthalmologic dosage forms. Table 18.7 includes the polymers used for making eye drops viscous – increasing the bioavailability of the preparations considerably.

Alginic acid [128] and Carbopol, and also the Carbopol-Pluronic combination [129, 130] are especially suitable for ensuring a sustained effect.

New possibilities in the medicinal treatment of the eye based on the use of polymers are inserts [6], matrix-membrane combinations with controlled drug

Table 18.7 Viscosity-increasing polymers in ophhtalmology (Trade named Preparations) [6].

Polymer	Active agents	Trade name
Hydroxyethylcellulose (HEC)	Azidamfenicol	Thilocanfol
	Dexamethasone/Neomycin	Chibro-Cadron
	Dorzolamide	Trusopt
	Guanethidine	Suprexon
	Guanethidine/Dipivefrine	Thilodigon
	Oxybuprocine	Thilorbin
Hydroxypropylmethylcellulose (HPMC)	Antazoline	Spersallerg
	Atropin	Atropin Dispersa, Atropin-EDO
	Carbachol	Isopto, Carbachol,
	Chloramphenicol	Jestryl
	Clonidin	Dispapheniol
	Cromoglicin acid	Cloniol-Ophtal
	Dapipral	Cromo von ct
	Dexamethason	Remydrial
	Dexamethason/ Chlopamphenicol	Spersadex, Isopto-
	Tetryzoline	Dex
	Dexamethason/Neomycin	Spersadex comp
	Polymyxin B	Spersadexolin
	Gentamycin	Dispadex
	Hydrastinin/Oxedrin	Isopto-Max
	Medryson	Dispagent
	Pilocarpin	Dacrin

(Continued)

Table 18.7 (cont.) Viscosity-increasing polymers in ophhtalmology (Trade named Preparations) [6].

Polymer	Active agents	Trade name
	Pilocarpin/Physostigmin Prednisolon Prednisolon/Sulfacetamid Retinolpalmitat Tetryzolin Timolol	Opptocortin Isopto Pilocarpin Isopto Pilomin Inflanefran Blefcon Oculotect, Solan-M Berberil, Vidiseptal Dispatim
Methycelluloe (MC)	Dexamethason	Dexapos
Sodium carboxymethylcellulose	8-Hydroxy-1-methylchinolin	Chibro-Uvelin
Poly(vinyl alcohol) (PVA)	Befunolol Fluorometholon Levobunololen Prednisolon	Clauconex Efflumidex Vistagan, Liquifilm Predni-POS
Poly(vinyl pyrrolidone) (PVP)	Cyclopentolat Metipranolol	Cyclopentolat Betamann
Combination PVA/PVP PVP/HEC	 Oxymethazoline Trifluridine	 Vistoxyn Liquifilm TFT Thilo

release [5-8, 22] (Figure 18.22) and thermosensitive gels, which are liquid and can be dropped at room temperature, but are transformed into mucoadhesive gels at the temperature of the eye [8, 132].

18.3.5 Mucoadhesive Preparations in the Rectum and in the Vagina

Mucoadhesion was already used as part of medicinal therapy in rectal dosage forms longer than 100 years ago. Viscosity-increasing polymers were used in the rectal solutions already at the beginning of the last century, so that that the preparation would adhere well and would not flow back [133]. A new achievement in this field is the development of mucoadhesive suppositories [18].

Polymer containing preparations are increasingly more frequent in the medicinal treatment of the vagina, too: mucoadhesive gels, swelling gels,

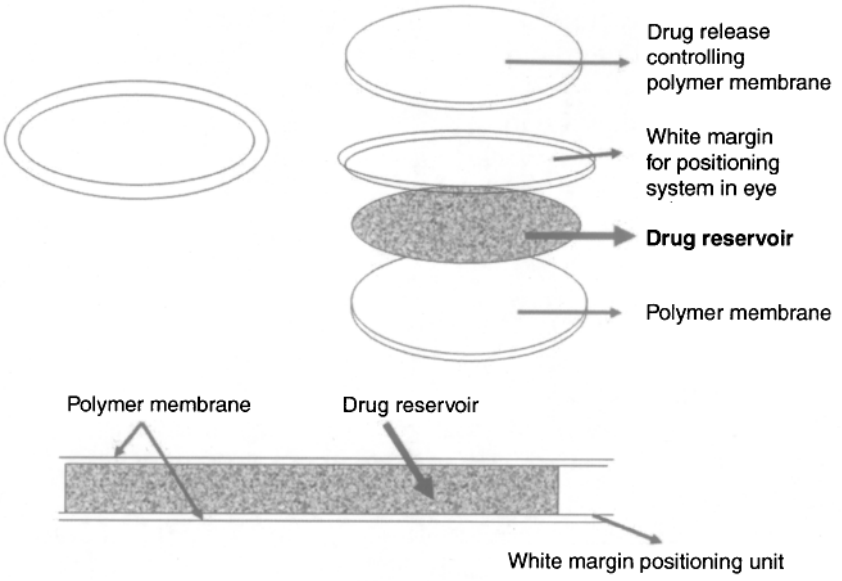

Figure 18.22 Schematic representation of Ocusert ocular therapeutic system showing functional elements [22].

thermoresponsive gels, nanoparticles, lipids, and liposomes are used in appropriate gels and polymer tampons [17, 134–141]. The purpose of the application is: i) to restore the pH and the microflora of the vagina, ii) to treat infections, iii) for local and systemic contraception, iv) to induce birth, and v) administration of the active substances into the systemic circulation through the mucosa of the vagina [17, 137, 140]. A wide range of polymers is used: alginates, chitosan, polyacrylates, cellulose derivatives, hyaluron acid and its derivatives, pectin, tragacanth, polyethylene glycol, carragenates, and gelatine [6, 140, 141].

18.4 Conclusion

Polymers and biopolymers are indispensable materials in pharmacy. The number and importance of polymer-structured active substances (e.g. enzymes, hormones, and antibiotics) are increasing continuously. Interesting references to auxiliary materials of polymeric character were already made as long ago as in the ancient written records on medicines, e.g. Ebers Papyrus, the Bible, or Greek epics. Karl Thoma, one of the most prominent researchers in this field, talked about the explosive increase of auxiliary materials used in modern drug preparations.

One characteristic quality change in this field is that the sharp distinction between active substances and additives has disappeared. The reason for this is that in controlled drug delivery systems the sustained, pulsatile, targeted drug release can almost be regarded as an independent therapeutic effect.

Polymers and biopolymers can not only be active substances but also preparation bases (e.g. ointment and suppository bases), stabilizing additives in emulsions and suspensions, fillers, binders, disintegrants, lubricants, and glidants in tablet preparation. Coating polymers lead us to a major field of modern pharmaceutical technology, which is controlling the release or dissolution of active substances.

Several possibilities are available to control drug effect. The most important ones are the following:

i. To accelerate drug release in the case of inadequate or slowly soluble active substances,
ii. to sustain drug release,
iii. to control drug release in space to ensure that the drug is released, for example, only in the small intestine and not in the stomach,
iv. to achieve a pulsatile drug release, meaning that the drug is released when it is necessary, for example, when the quantity of a given hormone decreases in the body, and
v. to target the active substance.

Drug release can be enhanced with the use of polymeric solvents and polymeric micelles. These ensure the molecular distribution of the active substance.

Drug release can be controlled with diffusion through the membrane or inclusion in a polymeric matrix. Biologically degradable polymers are considered to be a special controlling system. Pulsatile drug release can be ensured with the so-called environment-sensitive or stimuli-sensitive polymers. Active substances can be targeted with vesicles consisting of surfactant polymers.

A novel and important application method of polymers is the administration of active substances through the mucosa of body cavities, e.g. through the eyes, lungs or vagina. In these cases perfect adhesion, so-called mucoadhesion has to be ensured. Unfortunately, specific experiments of colloid chemistry and physiology are needed to select polymers for such purposes.

In summation, it can be said that the use of polymers and biopolymers is indispensable to modern pharmaceutical production and up-to-date medicinal therapy. This necessitates the thorough and detailed knowledge and experimental investigation of polymers.

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19

Biopolymers Employed in Drug Delivery

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Abstract

The use of natural raw materials is a worldwide tendency applicable in various industrial fields. In this regard, research involving biopolymers has attracted special interest in particular for pharmaceutical and cosmetic applications. Biopolymers represent an interesting alternative to synthetic polymers for use as material carriers for controlled release and encapsulation applications. These structures have the ability to entrap both hydrophilic and hydrophobic actives, and this is very promising for many applications. In addition, the absence of organic solvents used to produce biopolymeric matrices could be very interesting, especially in the pharmaceutical field. This chapter is organized according to the main contributions from literature concerning biopolymers used in drug encapsulation. The most commonly used biopolymers for the encapsulation of active compounds, as well as the encapsulation techniques are described in this chapter.

Keywords: Drug delivery, biopolymers, cellulose derivates, polysaccharides, polyhydroxyalcanoates, proteins

19.1 Introduction

The use of biopolymers in drug delivery systems was initially reported in the 80's, but only in the 21st century is it possible to observe an exponential increase in research involving this issue. Biopolymers represent an interesting alternative to synthetic polymers for use as structured carriers for controlled release and encapsulation applications, such as biofilms and particles preparation. These carriers have the ability to entrap both hydrophilic and hydrophobic drugs and may be very promising for many applications. Another advantage is the absence of chemical compounds and organic solvents used to produce biopolymeric matrices which could be very interesting for some industrial fields. For example, simple or complex coacervation methods involving proteins or protein and polysaccharide mixtures can be used to create new matrices dedicated to controlled-release applications [1]. The oral route of administration, which has long been the most convenient and commonly employed route of drug delivery for controlled-release applications, has received the most

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (559–574)

attention. Determination of the hydration rate of polymers used in sustained-release applications has been an area of interest because it is believed that the hydration rate affects drug release from controlled-release matrix. Hydration time is the time required for a polymer to reach maximum viscosity in a solvent. It has been proposed that faster hydrating polymers are more desirable because rapid gel development limits the amount of drug initially released from a matrix and further extends the period of release [2].

Polymeric nanoparticles have been widely investigated as pharmaceutical dosage forms because of their advantages, such as drug targeting, drug release control, improvement of drug efficiency, and reduction of drug toxicity. Polymeric nanoparticles comprise nanospheres and nanocapsules. The former are polymeric matrices whereas the latter are vesicular carriers composed of an oil core surrounded by a polymeric wall [3]. Both of these colloids are stabilized by surfactants at the particle/water interface [4]. Many methods are available for the preparation of polymeric nanoparticles using pre-formed polymers, such as nanoprecipitation and interfacial deposition [5], salting-out [6], emulsification-evaporation [7], and emulsification-diffusion [8]. The preparation of nanoparticles is also possible using the techniques of synthesis of biopolymers by methods such as suspension-polyaddition [9], interfacial polymerization, and miniemulsion [10]. The mean particle size and the particle size distribution (polydispersity) influence the drug release kinetics. Those characteristics depend on proceeding factors and formulation parameters, for example, the stirring rate, the type and concentration of the stabilizers, the polymer concentration, and the technique employed in nanoparticles preparation.

19.2 The Most Studied Biopolymers in Drug Delivery

One of the most important areas of research and development in drug delivery systems involves the development of controlled-release matrices, which may be defined as devices that deliver a therapeutic agent to a desired body location and/or provide timely release of such an agent. Polymers are typically the material of choice for such applications, given their diverse mechanical, physical, and chemical properties. Polymers may be either synthetic or natural in origin [11]. Those obtained from natural sources are generally referred to as biopolymers, and include various macromolecules such as gelatin, polysaccharides, and cellulose, among others. For controlled-release matrices, such materials exist as hydrated networks named hydrogels, where the water content accounts for up to ~98% of the total mass.

An analysis of the publications focused on the use of biopolymers in drug delivery systems shows that the first papers were published in the 80's (Figure 19.1). After 2000, the number of publications has become relevant, with an exponential increase from 2005. In 2010, the number of publications has already surpassed 500 according to the database Science direct. These data reflect the relevance of the topic and how promising the market is involving the use of biopolymers in drug delivery systems.

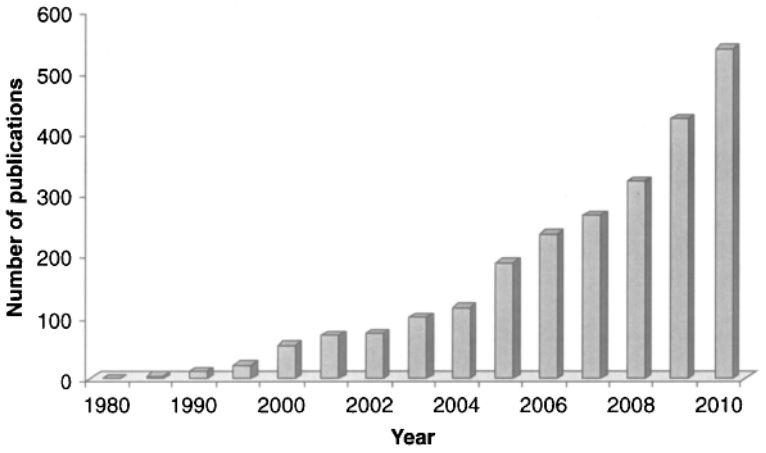

Figure 19.1 Number of scientific publications on the topic biopolymers in drug delivery fields in the last 30 years as a function of publication year.
Source: www.sciencedirect.com, available in October 2010.

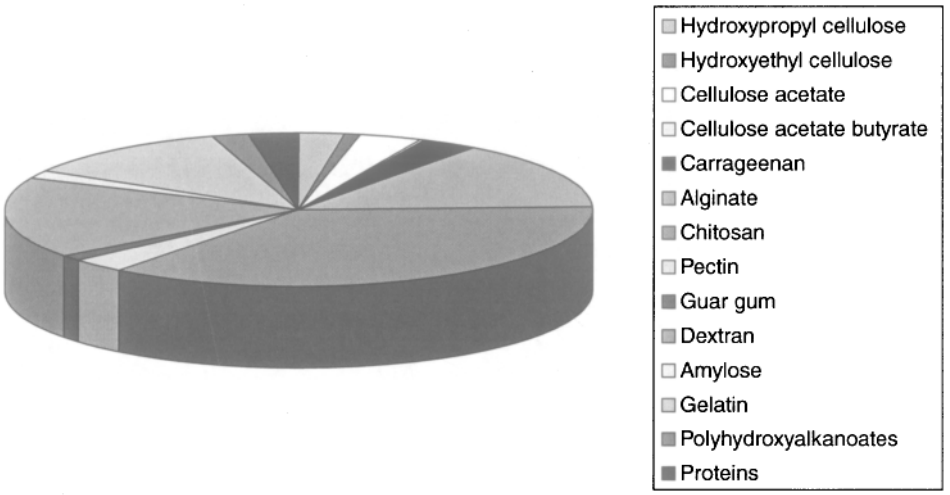

Figure 19.2 Scientific publications according to the type of biopolymer used in drug delivery in the last 30 years.
Source: www.sciencedirect.com, available in October 2010.

The most commonly used biopolymers in drug delivery are shown in Figure 19.2. Chitosan, biopolymer from crustaceous skin or shells, is the most studied for this application reported in 36% of the publications, followed by dextran (17%), alginate (14%) and gelatin (12%).

The properties and applications of each kind of biopolymer are described below.

19.2.1 Cellulose Derivatives

Cellulose derivative biopolymers have mucus adhesive properties and pH dependent solubility, which makes their use extremely interesting to delivery

systems for use in the gastrointestinal tract. With these biopolymers it is possible, for example, to choose a specific release of the drug in the stomach, intestine, or colon.

19.2.1.1 Hydroxypropyl Cellulose

Hydroxypropyl cellulose is a nonionic water-soluble cellulose ether with a remarkable combination of properties such as organic-solvent solubility, thermo plasticity, and surface activity. The molecular weight is varied by controlling the degree of polymerization (DP) of the cellulose backbone and this controls the viscosity of this polymer; as the DP increases, the viscosity of the polymer increases. Hydroxypropyl cellulose is used in pharmaceutical formulations for various purposes: low-viscosity grades are used as tablet binders in immediate-release dosage forms, and medium- and high-viscosity grades are used in sustained-release matrix formulations. The release rate of a drug increases with decreasing viscosity of the polymer. In oral pharmaceutical products, hydroxypropyl cellulose is primarily used in tableting as a binder, film coating, and for controlled-release matrix [2].

19.2.1.2 Hydroxyethyl Cellulose

Also called Natrosol® (250HX hydroxyethyl cellulose, Hercules, Wilmington, DE, USA), hydroxyethyl cellulose is a nonionic water-soluble polymer derived from cellulose. It can be used to produce solutions with a wide range of viscosity and its solution behaviour is unaffected by the presence of cations. Hydroxyethyl cellulose is of use in a range of pharmaceutical applications, such as a bioadhesive in mucoadhesive patches, a thickening agent in ophthalmic and topical formulations, a matrix controlled-release polymer in solid dosage forms, and as a binder and film-coating agent for tablets [12].

19.2.1.3 Hydroxypropyl Methylcellulose

O-methylated and O-(2-hydroxypropylated) cellulose is available in several grades that vary in viscosity and extent of substitution. It is widely used in pharmaceutical formulations: in film-coating and as a controlled-release matrix, in oral products and tablet binder (between 2–5% w/w), and in either wet or dry granulation processes. High-viscosity grades can be used to retard the release of water-soluble drugs from a matrix [2].

19.2.1.4 Sodium Carboxymethyl Cellulose

Sodium carboxymethyl cellulose is an anionic water-soluble polymer and is widely used in oral and topical pharmaceutical formulations because of its viscosity-increasing properties. Viscous aqueous solutions are used to suspend powders intended for topical, oral, or parenteral applications [13]. It is also used as a tablet binder and to stabilize emulsions [14].

19.2.1.5 Cellulose Acetate

Cellulose acetate (CA), the acetate ester of cellulose, is one of the most commonly used biocompatible materials for the preparation of semi-permeable membranes to be used for dialysis, ultrafiltration, and reverse osmosis. CA membranes have very low absorption characteristics and thermal stability with high flow rates. Cellulose-based materials are also widely used in the biopharmaceutical industry as the matrix for adsorbent beads and membranes. Moreover, CA nanofibers can be used as carrier for delivery of vitamins or pharmaceutical products [15].

19.2.1.6 Cellulose Acetate Butyrate

Cellulose acetate butyrate is insoluble in water and is available in several viscosity grades depending on their molecular weight. This polymer has been used to obtain sustained-release matrices prepared by direct compression technique as well as in obtaining semi-permeable membranes for osmotic pump systems [16]. Cellulose acetate butyrate microparticles (Figure 19.3) for the sustained release of drugs can be obtained by the emulsion-solvent evaporation method [17].

19.2.2 Biopolymers from Marine Source

19.2.2.1 Carrageenan

Carrageenan is a linear polysaccharide; more specifically, it is a galactan with galactose residues. This polymer is obtained by extraction with water or alkaline water of certain species of the class Rhodophyceas (red seaweeds). Because

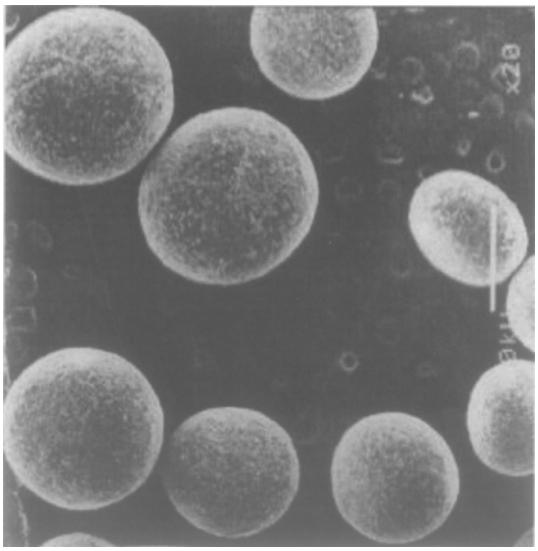

Figure 19.3 Cellulose acetate butyrate microparticles for drug delivery systems.

carrageenan is a large molecule made up of some 1000 residues, it is evident that the possibility for structural variations is enormous. There are three main types: kappa, iota, and lambda carrageenans. In aqueous solutions and in the presence of cations, carrageenans form thermoreversible gels, and they can provide stability by modifying the rheological properties of the continuous aqueous phase [18]. They are widely used in different pharmaceutical formulations for their stabilizing, thickening, and gelling properties [2].

19.2.2.2 Alginate

Alginate (Figure 19.4) is a water soluble linear polysaccharide extracted from some types of brown algae (alginate comprises up to 40% of the dry weight), and it is made up of two uronic acids: d-mannuronic acid (M) and l-guluronic acid (G). Polyvalent cations are responsible for inter- and intrachains crosslinking because they are tied to the polymer when two guluronic acid residuals are close [2]. Alginate can be ionically crosslinked by the addition of divalent cations in aqueous solution. It was reported that alginate is nontoxic and biodegradable when given orally [19]. Alginates, have received great attention for use in pharmaceutical dosage forms, particularly as a vehicle for controlled drug delivery. The formation of a matrix upon hydration causes a gelatinous layer which can act as a drug diffusion barrier. For the encapsulation process, alginate shrinks at low pH and the encapsulated drugs are not released. The biological activity of drugs can be retained in the calcium-crosslinked alginate encapsulation process [19]. The biocompatibility of alginate is well covered in literature because this biopolymer may be used in implanted devices [20]. In delivery systems, alginate fibres reveals biphasic behaviour, featured by an initial burst followed by a slower and time sustained release phase. This feature becomes useful for those treatments where a strong therapeutic initial dose is required, followed by a maintenance therapy with lower doses, e.g. for standard treatment of inflammatory diseases of the CNS (such as lupus cerebritis, vasculitis, and multiple sclerosis) [21].

19.2.2.3 Chitosan

Chitosan is a cationic polymer, which is the second most abundant polymer in nature after cellulose and biopolymer, the most widely used in drug delivery. Chitosan is a linear copolymer polysaccharide consisting of β (1–4)-linked 2-amino-2-deoxy-D-glucose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) units (Figure 19.5). Chitin is the primary

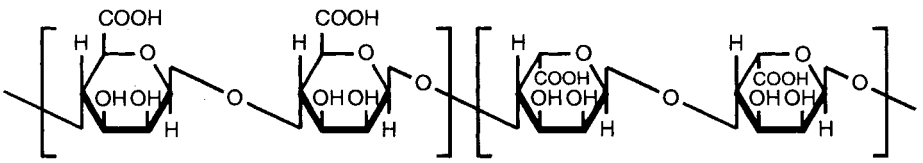

Figure 19.4 Structure of alginate.

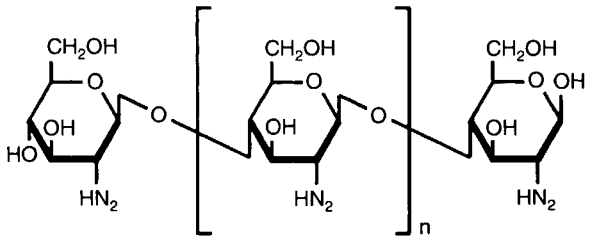

Figure 19.5 Structure of chitosan.

structural component of the outer skeletons of crustaceans, and is also found in many other species such as molluscs, insects, and fungi. The most commonly obtained form of chitosan is the α-chitosan from crustacean chitin obtained from crab and shrimp shell wastes. This accounts for approximately 70% of the organic compounds in such shells. In preparing chitosan, ground shells are deproteinated and demineralized by sequential treatment with alkali and acid, after which the extracted chitin is deacetylated to chitosan by alkaline hydrolysis at high temperature. Production of chitosan from these sources is inexpensive and easy. Chitosan as such, is rare in nature except in certain fungi, and recently the production of chitosan from fungi using fermentation methods also has intensified. Chitosan is soluble in dilute acid and precipitates at a pH above 7. Because of the solubility of chitosan at low pH ranges, its successful use in colon-specific delivery requires an enteric layer over the chitosan which protects it against the acidity of the stomach. As the formulation reaches the intestine, the pH increases and the enteric layer dissolves releasing the chitosan coated core. These cores are acted upon by microflora of the colon, degrading the chitosan and releasing the drug [22].

The chitosan has mucoadhesive properties and could be useful in site-specific drug delivery. It has been suggested that chitosan might be valuable for the delivery of drugs to specific regions of the gastrointestinal tract the stomach, small intestine and buccal mucosa [23]. Moreover, chitosan has favourable biological properties such as nontoxicity, biocompatibility, and biodegradability.

19.2.3 Others Polysaccharides

19.2.3.1 Pectin

Pectin is a structural cell wall, non-starch, and linear polysaccharide carbohydrate present in all higher plants obtained by aqueous extraction of appropriate edible plant material, usually citrus fruit or apples. Pectin is essentially a linear polysaccharide containing from a few hundred to approximately 1000 sugar units in a chain-like configuration; this corresponds to an average molecular weight from approximately 50,000–150,000 (Figure 19.6). The main property of pectins is their ability to form gels in the presence of calcium ions or sugar and acid, making them an important ingredient of many food and pharmaceutical

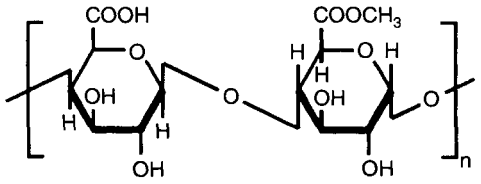

Figure 19.6 Structure of pectin.

products. Pectin has many applications within the pharmaceutical industry. When injected intravenously, pectin shortens the coagulation time of drawn blood, thus making it of use in the control of haemorrhage or localized bleeding. Additionally, it has been reported to help reduce blood cholesterol [24]. These polysaccharides remain intact in the physiological environment of the stomach and the small intestine, but are degraded by the bacterial inhabitants of the human colon. However, being soluble in water, pectin is not able to shield its drug load effectively during its passage through the stomach and small intestine and a thick coating is necessary to protect the drug core in simulated *in vivo* conditions [25].

19.2.3.2 Guar Gum

Chemically, guar gum is a galactomannan, best described as a galactose on every other mannose unit (Figure 19.7). It is obtained from the ground endosperm of the guar plant (*Cyamopsis tetragonolobus*), which is grown in India, Pakistan, and the semi-arid southwestern region of the USA. Guar gum is a nonionic polysaccharide consisting of a (1-4)-linked-d-mannopyranose backbone with branch points from their 6-positions linked to -d-galactose (i.e. 1-6-linked-d-galactopyranose) and containing about 80% galactomannan, 12% water, 5% protein, 2% acid insoluble ash, 0.7% ash, and 0.7% fat. That is, it consists of linear chains of (1→4)-d-mannopyranosyl units with-d-galactopyranosyl units attached by (1→6) linkages. It can disperse and swell almost completely in cold or hot water to form a viscous sol or gel. The hydration rate and optimum viscosity of guar gum are strongly affected by the galactomannan content, the molecular weight of the polymer, and its particle-size distribution. Guar gum has the ability to develop extremely high viscosity, even at low concentration. In pharmaceuticals, it is used in solid dosage forms as a binder and disintegrant, and in liquid oral and topical products as a suspending, thickening,

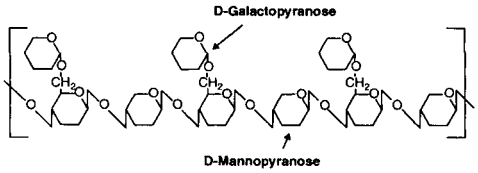

Figure 19.7 Structure of guar gum.

and stabilizing agent. Guar gum has been used in colon-specific drug delivery as matrix forming material and as a compression coat [26]. In therapy, guar gum has been used as part of the diet of patients with diabetes mellitus. Guar gum is also used as a thickener in cosmetics, sauces, salad dressings, and as an agent in ice cream that prevents formation of ice crystals. Guar gum is a potential hydrophilic matrix carrier for oral controlled delivery of drugs with varying solubility [19], and is used to deliver drugs to the colon due to its drug release retarding property and susceptibility to microbial degradation in the large intestine.

19.2.3.3 Dextran

Dextrans (Figure 19.8) are a class of polysaccharides with a linear polymer backbone with mainly 1,6-D-glucopyranosidic linkages. They are obtained from bacterial cultures of *Leuconostoc mesenteroides* and represent the second group of biopolymers most studied in drug delivery with 17% of publications. These glycosidic linkages are hydrolysed by moulds, bacteria, and by the mammalian cells. Dextranases are the enzymes which hydrolyse these glycosidic linkages. Various drug-dextran prodrugs in which the drug molecule is linked to the polar dextran macromolecule remain intact and are absorbed from the stomach and the small intestine, but when the prodrug enters into the colonic microflora containing Bacteroides it is acted upon by dextranases which cleave the dextran chain randomly and at the terminal linkages, releasing the drug freely into the colon [25].

19.2.3.4 Amylose

Amylose is a polysaccharide from plant extracts and a component of starch. It consists of D-glucopyranose residues linked by α(1-4) bonds. It is a poly(1,4′ α-D–glucopyranose) (Figure 19.9). They have the ability to form films that are water swellable and are potentially resistant to pancreatic α-amylase [27], but

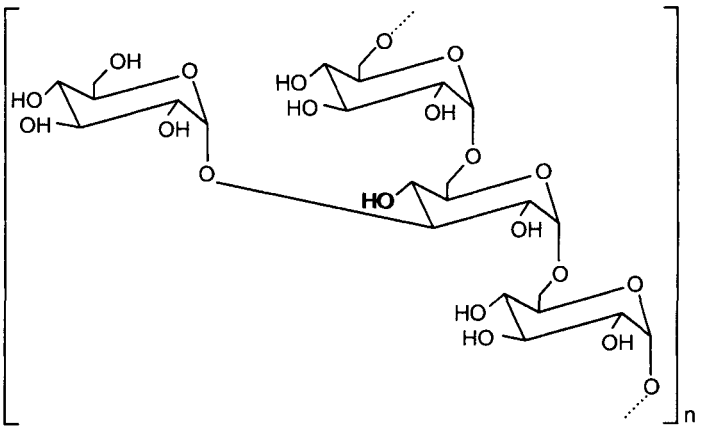

Figure 19.8 Structure of Dextran.

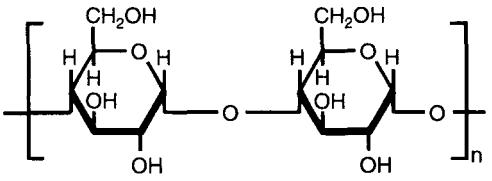

Figure 19.9 Structure of amylose.

are degraded by colonic bacterial enzymes. A commercial product Amylose-Ethocel® is a coating system resistant to gastric acid and small intestinal enzymes, but degradable by colonic bacteria. A coating formulation comprised of Amylose and Ethocel® in the ratio of 1:4 w/w shows optimum drug release retarding properties in gastric and intestinal fluids [28, 29].

19.2.3.5 Starch

Starch is one of the most promising natural polymers because of its inherent biodegradability, overwhelming abundance, and renewability. It is composed of a mixture of glycans that plants synthesize and deposit in the chloroplasts as their principal food reserve. Starch is stored as insoluble granules composed of α-amylose (20–30%) and amylopectin (70–80%) [30]. α-amylose is a rather linear polymer of several thousands of glucose residues linked by α(1→4) bonds with some α(1→6) links. The α-glycosidic bonds of α-amylose cause it to adopt a helical conformation (left-handed helix). Amylopectin consists mainly of α(1→4)-linked glucose residues but it is a branched molecule with α(1→6) branch points every 24 to 30 glucose residues in average. Amylopectin molecules contain up to 106 glucose residues, making them some of the largest molecules in nature. Starch by itself is extremely difficult to process and is brittle when used without the addition of a plasticizer. In most applications, the semi-crystalline native starch granule structure is either destroyed or reorganized, or both. Water is the usual plasticizer in starch processing, and the physical properties of starch are greatly influenced by the amount of water present. The use of other plasticizers, such as low molecular weight alcohols, especially for the production of thermoplastic starches, renders starch more processable [31]. Over the years several materials have been blended with starch to improve its processability, including polymers such as polyethylene, polycaprolactone, polyethylene-co-vinyl alcohol, poly(hydroxybutyrate-co-valerate), polysaccharides, and proteins [32]. Starch has also been extensively modified by chemical methods such as oxidation and grafting of acryl reactive groups. Starch has been extensively used for drug delivery applications, including cancer therapy, nasal administration of insulin, and a wide range of biomedical applications, such as scaffolds for bone tissue engineering applications and drug delivery systems [33]. Destructured starch has been shown to be biocompatible *in vitro*, possess a good *in vivo* performance and permit the adhesion of endothelial cells, an indicator of the ability of starch-based fibre scaffolds to permit vascularisation to occur [34].

19.2.4 Polyhydroxyalcanoates

The polyhydroxyalkanoates (PHAs) are a family of bacterial polyesters derived from microorganisms, especially *Escherichia coli K12*. In nature, these polyesters are produced inside cells as storage granules and regulate energy metabolism. The use of *E. coli K12* has several advantages; it has many applications in the biopharmaceutical industry, is well understood, is highly efficient, and is widely used to produce products for human use. The yields of poly-4-hydroxybutyrate (P4HB) exceed 50 g per litre of fermentation broth in less than 48h, making large-scale production attractive. The structure of P4HB (Figure 19.10) strongly resembles that of chemically derived polyesters. However, because it is biologically produced, it does not contain residual metal catalysts that are used in the chemical synthesis of other polyesters [35].

The polyalkanoates are of great commercial interest because of their unique properties and relative ease of production. The P4HB is used as a new absorbable biomaterial for medical applications. In the field of cardiovascular research, for example, the use of P4HB has resulted in the first successful demonstration of a tissue engineered tri-leaflet heart valve in a sheep model. Other products under development include vascular grafts, stents, patches, and sutures. This biopolymer promises new opportunities for the development of medical applications by offering a new set of properties that are not currently available. The absorbable biomaterial is strong yet flexible, and degrades *in vivo* at least in part by a surface erosion process. Also, the chemical structure of P4HB is similar to that of current absorbable polyesters used in implantable medical products [35]. P4HB is also used in wound healing, orthopaedic and drug delivery. Poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) copolymer is attractive for use in biomedical devices due to its adequate biocompatibility, biodegradability, and thermoprocessability [36]. Although the PHBV chemical structure is very similar to the highly popular synthetic biodegradable polymers, such as PLA and PLGA, it generally degrades at a much slower rate [37]. PHBV is highly soluble in chloroform or dichloromethane, and presents poor solubility in other solvents, such as ethyl acetate. Under such solubility conditions, particles for drug delivery can be easily obtained by emulsification-solvent evaporation [38]. The preparation of PHBV nanoparticles has been described by several techniques: double emulsion-solvent evaporation procedure using dichloromethane [39], thermo reversible gelation of PHBV in toluene [40], and nanoparticles of PHBV prepared by emulsification-diffusion technique using ethanol as a surface agent [41].

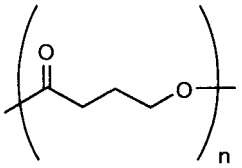

Figure 19.10 Chemical structure of poly-4-hydroxybutyrate (P4HB).

19.2.5 Biopolymers from Proteins

19.2.5.1 Gelatin

Gelatin is a natural polymer that is derived from collagen, and is commonly used for pharmaceutical and medical applications because of its biodegradability and biocompatibility in physiological environments [42]. Two different types of gelatin can be produced depending on the method in which collagen is pretreated, prior to the extraction process. The alkaline process targets the amide groups of asparagines and glutamine, and hydrolyses them into carboxyl groups, thus converting many of these residues to aspartate and glutamate. To the contrary, acidic pretreatment does little to affect the amide groups and the gelatin processed with an alkaline pretreatment is electrically different in nature from acidic-processed gelatin. The alkaline processed gelatin possesses a greater proportion of carboxyl groups, rendering it negatively charged and lowering its isoelectric point (IEP) compared to acidic-processed gelatin which possesses an IEP similar to collagen. By utilizing this technique, manufacturers now offer gelatin in a variety of IEP values. Gelatin has a proven record of safety as a plasma expander, as an ingredient in drug formulations, and as a sealant for vascular prostheses [43]. In addition, its biodegradability, biocompatibility, nontoxicity, ease of chemical modification, and cross-linking are used to make gelatin-based particles (Figure 19.11) for many applications in drug delivery.

19.2.5.2 Wheat Gluten

Wheat gluten is a protein carbohydrate complex of which proteins are the major component. Two main fractions are present: gliadin, which is soluble in neutral 70% ethanol, made of single chain polypeptides with an average molecular

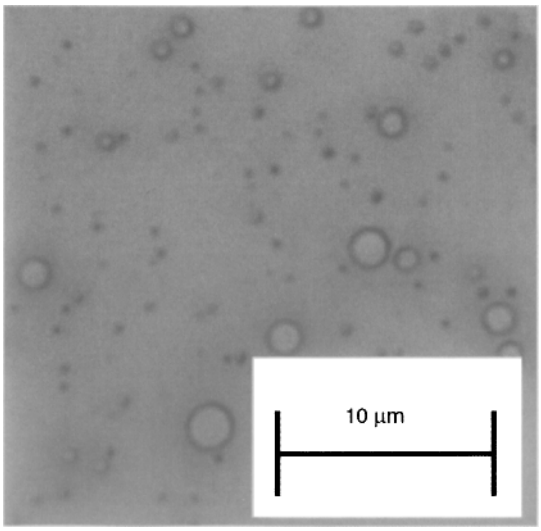

Figure 19.11 Optical microscopy of gelatin microparticles.

weight of 25–100 kDa linked by intramolecular disulphide bonds; and glutenin, an alcohol-insoluble fraction consisting of gliadin-like subunits stabilized by intermolecular disulphide bonds in large aggregates with molecular weight greater than 106 kDa [44]. Thus, the term gliadin defines a group of proteins extracted from gluten by 70% ethanol [45]. These proteins are polymorphic and can be classified on the basis of their electrophoretic mobility in four fractions, named alpha (molecular mass about 25–35 kDa), beta (30–35 kDa), gamma (35–40 kDa), and omega (55–70 kDa) respectively [46]. All fractions have low solubility in aqueous solution except at extreme pH. This low water solubility has been attributed to the presence of interpolypeptide S-S bonds and to the cooperative hydrophobic interactions which cause the protein chains to assume a folded shape. Gliadins nanoparticles can be produced by simple coacervation obtained by desolvation of the protein using physiological salt solution as a nonsolvent [1]. Moreover, due to the low solubility of this protein in water and to its high hydrophobicity, nanoparticles from gliadin do not need any further chemical or physical treatment to harden them. Nanoparticulate carriers from vegetal macromolecules are a new approach which may present some advantages. Proteins are metabolizable and they can incorporate a wide variety of drugs in a relatively nonspecific fashion. Moreover, it has been reported that some exogenous proteins (e.g. gliadin from wheat gluten) are able to interact with epidermal keratin of the skin by means of weak but numerous bonds [47].

19.3 Conclusion

Natural origin polymers have received considerable interest for drug delivery systems. This chapter reviewed the properties of natural-origin materials employed in drug delivery and the great interest in scientific research about this topic, especially in the last 4 years. The wide variety of biopolymers and their biocompatibility and biodegradability characteristics, in addition to the potential application of the materials made from them, make them highly promising for commercial use in drug delivery fields. Additionally, aspects related to the use of environmentally friendly materials and use of safe materials of natural composition, as is the case of biopolymers, will make the difference when choosing an encapsulating system for drug delivery and other applications.

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20

Natural Polymeric Vectors in Gene Therapy

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Abstract

Viral vectors, liposomes, and synthetic polymeric vectors are the most widely used gene carriers in gene therapy. But some unique properties, such as biodegradability, biocompatibility, and low toxicity of natural polymers advance them to use as nonviral vectors in gene therapy. Among the natural polymers, chitosan and their derivatives are the strong candidates as nonviral vectors in gene therapy due to their reduced cytotoxicity, biodegradability, excellent biocompatibility, and low immunogenicity character. It has been successful in oral and nasal delivery due to its mucoadhesive property. Apart from chitosan, gelatin, collagen, arginine, and alginate are also used as gene carriers. The natural polymers can be tailored through ligand conjugation, cross-linking and many other modifications with its reactive sites and used for a wide range of clinical applications. Due to the gene carrier ability of natural polymers, they can play an important role in the field of regenerative medicines. This chapter highlights the present and past research on natural polymers as nonviral vectors in gene therapy.

Keywords: Gene therapy, natural polymers, nonviral vectors, biocompatibility, cytotoxicity

20.1 Introduction

In the present day, the modern clinical treatment tends to shift towards tailored treatments that include higher specificity and minimum side effects. The next generations of therapeutics, such as proteins and nucleic acids, have been considered to fulfill the needs of modern treatment. Due to the poor bioavailability, rapid clearance *in vivo*, cytotoxicity and higher manufacturing cost, recombinant protein drugs have been limited in their clinical applications [1]. Recently, gene therapy has been recognized as an alternative pathway to overcome the drawbacks of protein therapy. Over the past two decades, gene therapy has gained significant attention as a potential method for treating genetic disorders, such as cystic fibrosis [2], severe combined immunodeficiency [3], Parkinson's disease [4], hemophilia [5], muscular dystrophy [6–8], cardiovascular diseases [9], neurological diseases [10–12], wound healing [13], as well as cancer [14–16]. The basic concept of gene therapy is the transfer of genetic

Susheel Kalia and Luc Avérous (eds.) Biopolymers: Biomedical and Environmental Applications, (575–604)

material into specific cells of a patient to correct or replace defective genes responsible for genetic disease development [17]. As most of the genetic materials (nucleic acids) are susceptible to enzymatic digestion by nucleases during the intracellular transfer, appropriate carriers are required for efficiently delivering nucleic acids to the specific cells to get maximum therapeutic effects.

In general, there are two types of carriers or vectors in gene therapy, such as (i) viral vectors and (ii) non-viral vectors. Initially, researchers concentrated on viral vectors including retroviruses, adenoviruses and adeno-associated viruses, because the viral vectors exhibited higher transfection efficiency of both DNA and RNA to numerous cell lines [18]. However, some drawbacks of viral vectors, including higher toxicity, lack of specificity towards target cells, risk of potential immunogenicity (safety concerns), higher production cost, and chromosomal insertion of viral genome, limit its clinical applications [19]. In addition, an immune reaction against adenovirus occurred in a patient at Pennsylvania University in 1999 [20, 21]. As a result, the researchers show much more interest towards the nonviral vectors. Although nonviral vectors exhibit significantly reduced transfection efficiency due to hindrance of numerous extra- and intracellular obstacles (Figure 20.1), biocompatibility, lower toxicity, simpler large scale production with low cost, and low host immunogenicity make them much more attractive in gene therapy [22]. Cationic lipids, polymers, dendrimers and peptides are considered as nonviral vectors in gene therapy for their potential of compacting DNA for systemic delivery.

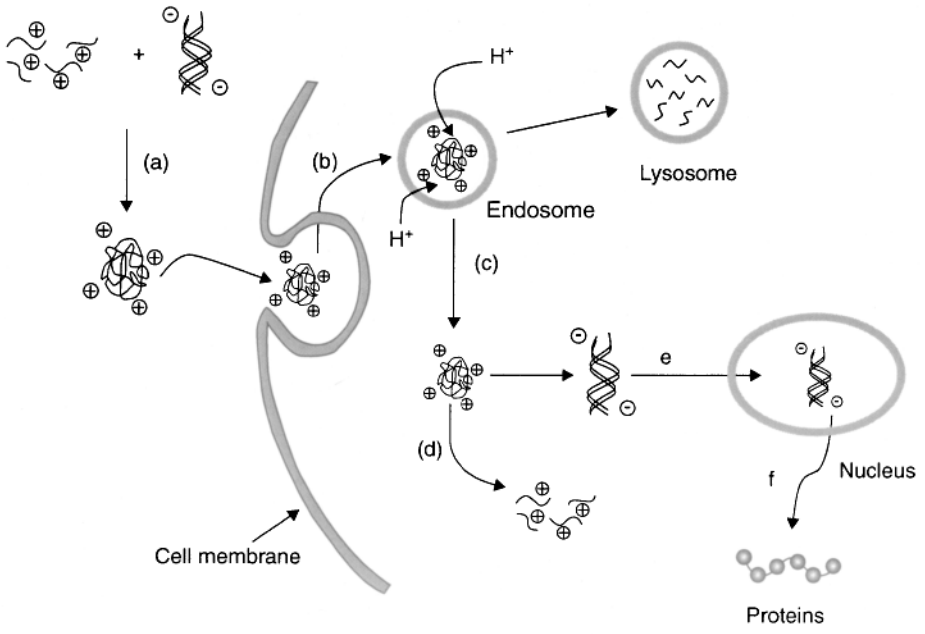


Figure 20.1 Barriers to gene delivery – Design requirements for gene delivery systems include the ability to (a) package therapeutic genes; (b) gain entry into cells; (c) escape the endo-lysosomal pathway; (d) effect DNA/vector release; (e) traffic through the cytoplasm and into the nucleus; (f) enable gene expression; and remain biocompatible.

There is increasing attention towards the nonviral vectors for the application in a broad variety of gene mediated therapy for humans. In the pharmaceutical industry, chemical vectors are more attractive as alternatives to viral vectors due to compound stability and easy chemical modification. To get specific therapeutic action, the nonviral vectors need to be designed in such a way that they must be able to partially or totally fulfill a number of predetermined biological criteria, such as (i) they must be able to transfer DNA molecules and to protect DNA from digestion by nucleases, (ii) they should target a specific cell type, (iii) they should have no or minimum toxicity *in vivo* and avoid exciting the immune system, (iv) they should be able to achieve sustained expression over a defined period of time (depending on therapeutic application), and (v) they must not renovate the target cell.

It is reported that cationic phospholipids and cationic polymers are the two major types of nonviral vectors in gene therapy. They can easily form complexes (lipoplexes and polyplexes) with the negatively charged DNA due to the presence of their permanent cationic charge. There is limited success of liposomal approach by cationic phospholipids, although they have several advantages, like low immunogenicity and ease of preparation over the viral vectors [23]. But, cationic lipids also have some disadvantages, including toxicity and relatively low transfection efficiency compared to viral vectors [24]. On the other hand, the cationic polymers form more stable complexes with DNA than those of cationic lipids [25]. The gene delivery efficiency of cationic polymers is found to be relatively low compared to the viral vectors [26].

This chapter will discuss the current status of the use of natural polymers as nonviral vectors for gene delivery. Although synthetic polymers are the major gene delivery systems, natural polymers have the distinct advantages, including the intrinsic property of environmental responsiveness via degradation and remodeling by cell-secreted enzymes over the synthetic polymers. Natural polymers can readily be incorporated *in vivo* because they are nontoxic at high concentration and biocompatible.

20.2 Cationic Polymers

Cationic polymers used as nonviral vectors in gene therapy can be classified in two groups, (i) natural polymers, including chitosan, gelatin, collagen, arginine, alginate, and (ii) synthetic polymers, such as polyethyleneimine (PEI), dendrimers, poly(L-lysine) (PLL) and polyphosphoester [27–29]. The cationic polymers can easily be associated with negatively charged DNA by electrostatic interaction due to the presence of protonable amine residues in its structures. The effective diameter and zeta potential of the complexes largely depend on the ratio of amines in the cationic polymers to phosphate groups on the plasmid, often referred to as the N/P ratio [30]. The advantage of the cationic polymers over the cationic lipids is that they do not contain a hydrophobic moiety and are completely soluble in water [31]. Compared with cationic liposomes, they can form relatively small complexes with DNA [32, 33].

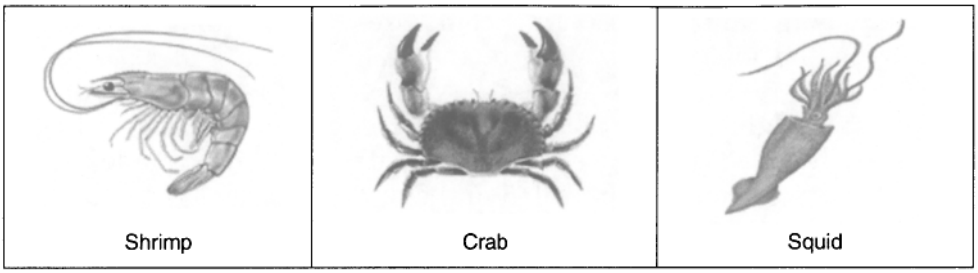
This factor is more important for gene transfer because small particle size may be more favorable for improving transfection efficiency. It is also found that high molecular weight polymers tend to form more stable and small complexes compared to low molecular weight polymers, although the transfection efficiency can be increased with low molecular weight polymers, likely due to a lowered cytotoxycity and the increased ability of the dissociation of the plasmid from the cationic polymers [34, 35]. Therefore, there is enough scope to improve the transfection efficiency and target specificity of these polymers through chemical modifications, such as change in molecular weight, geometry (linear vs. branched) and ligand attachment [36, 31]. The most commonly used cationic polymers in gene therapy are PEI, PLL, polyamidoamine (PAMAM) dendrimer, chitosan, polyphosphoesters, gelatin, etc.

20.3 Natural Polymers as Nonviral Vectors in Gene Therapy

20.3.1 Chitosan

Natural cationic polymers have several advantages, including nontoxicity, biodegradability, biocompatibility, and low immunogenicity over the synthetic cationic polymers. Chitosan and their derivatives are the strong candidate among the other natural polymers used as nonviral vectors in gene therapy due to their reduced cytotoxicity, biodegradability, excellent biocompatibility, and low immunogenicity character. In addition to this, chitosan and their derivatives can effectively be complexed with the negatively charged DNA due to its positive character, and can also protect the DNA from nuclease degradation [37–40]. Chitosan has other added advantages such as there is no necessity of sonication and organic solvents during the complex formation with DNA, therefore lowering possible damage of DNA during complexation. It is also found that DNA-loaded chitosan microparticles remain stable during storage [41]. Chitosan has mucoadhesive property which permits a sustained interaction between the "delivered" macromolecule and the membrane epithelia for more efficient uptake [42–44], and it also has the ability to open intercellular tight junctions which facilitate its transport into the cells [45].

Chitosan is a naturally occurring linear binary cationic polysaccharide consisting of 2-acetamido-2-deoxy-D-glucopyranose (acetylated unit) and 2-amino-2-deoxy-D-glucopyranose (deacetylated unit) units linked by a β (1→4) glycosidic bond at varying ratios and of varying chain lengths. It is obtained by the deacetylation of chitin, which is obtained from crab and shrimp shells by chemical processing (Scheme 20.1). Chitosan is a weak base due to the pK_a value of the D-glucosamine unit of about 6.2–7.0. So, it is insoluble at neutral and alkaline pH values. But, it is soluble in acidic medium because the amine groups of the chitosan become positively charged leading to the polysaccharide having a high charge density [46]. The transportation of drugs across the cell membrane is excellently enhanced by chitosan due to its cationic polyelectrolyte nature, which provides a strong electrostatic



Shellfish wastes from food processing (shrimp, crab, squid)

↓

Decalcification with 3% to 5% HCl solution (w/v) at room temperature

↓

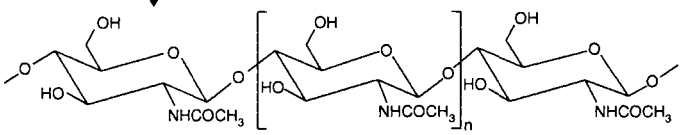
Deproteination with 3% to 5% NaOH solution (w/v) at 80°C to 90°C for few hrs.

↓

Decolorization with 0.5% aqueous $KMnO_4$ solution and aqueous oxalic acid solution

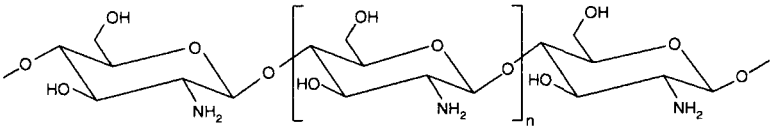
↓

Chitin



↓ Deacetylation with 40% to 50% (w/v) NaOH solution at 90°C to 120°C for 4 to 5 hrs

Chitosan



Scheme 20.1 Preparation of chitin and chitosan.

interaction with negatively charged mucosal surfaces of mucus as well as other macromolecules like DNA [46, 47]. Chitosan is not only used for gene delivery but also it is used as a delivery tool for nasal, ocular, and peroral drug delivery [48].

Self-assembled polymeric and oligomeric chitosan/DNA complex is formed by mixing chitosan with plasmid DNA was first reported by Mumper *et al.* in

1995 [49]. They found that sizes of the complexes (150–500 nm) were largely depended on the molecular weight of the chitosan (108–540 kDa). Other authors also reported that the sizes of the complexes not only depended on the molecular weight of chitosan but also on the ratio of chitosan and DNA [50, 51]. Erbacher *et al.* [52] showed that a comparatively large complex of chitosan/DNA formed (ranging from 1–5 μm) at a N/P ratio [as the number of polymer nitrogen (N) per DNA phosphate (P)] with a zetapotential close to 0 mV, indicating full retardation of plasmid DNA and aggregation of complexes that occurred due to neutral complex charge. Mao *et al.* [53] prepared chitosan/DNA nanoparticles by a complex coacervation method by using sodium sulphate as desolvating agent and got particle sizes ranging from 200–500 nm. In a recent study, they showed that there were several parameters that could influence the particle size of the chitosan/DNA complexes [54]. They found that the concentration of sodium sulphate (ranging from 2.5–25 mM) did not have any effect on the particle size, but the particle size depended strongly on the N/P ratio. They obtained particle sizes ranging from 150–250 nm at N/P ratios between 3 and 8 during the preparation of particles at a temperature of 55°C and a pH of 5.5. They also observed that the size of the plasmids (ranging from 5.1–11.9 kb) had no effect on the particle sizes of the complexes.

Due to the cationic nature of chitosan, it can bind with negatively charged DNA by electrostatic interaction, which can be determined by competitive binding tests using ethidium bromide (EtBr) as a DNA stain [54]. As chitosan is added to a solution of EtBr-stained DNA, cationic chitosan binds with DNA which causes a decrease in fluorescence, determined by confocal laser scanning microscopy [55]. There are various methods for the determination of the extent of DNA complexation or encapsulation into chitosan nanoparticles, such as gel electrophoresis [54], Pico Green assay [55], and photoelectric methods.

Transfection efficiency of chitosan/DNA complexes depends on several factors, such as molecular weight of chitosan, stoichiometry of chitosan/DNA complex, serum concentration and pH of the transfection medium [56, 57]. The mechanism of transfection with respect to the properties of the polymer is still not clear. In a study, MacLaughlin *et al.* [50] worked with chitosan of molecular weight ranges from 7–540 kDa and obtained the highest expression level of the pGL3-Luc gene with the 540 kDa chitosan in the presence of serum, but in absence of serum, the result was better with the 102 kDa chitosan (Figure 20.2). However, they were unable to provide a convincing explanation of the relation between the molecular weight of chitosan and gene expression. There is another important structural parameter, degree of deacetylation (DDA) of chitosan, which also has an important role in the gene expression. Various polymers' properties, like charge density (associated with the number of primary amine groups), solubility, crystallinity, and degradation rate [58–62]. Kiang *et al.* [63] showed that the chitosan with lower DDA can efficiently bind the DNA with higher N/P ratios, but *in vitro* gene expression into different cells (HEK 293, SW 756, and HeLa) was observed to decrease (Figure 20.3). The decrease in gene expression with lower DDA of chitosan is due to the instability of chitosan/DNA complexes by serum protein interactions. In another study,

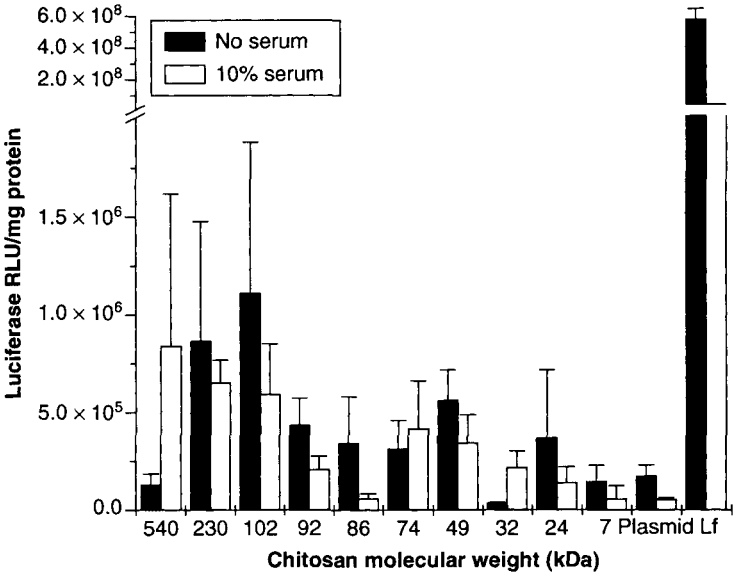


Figure 20.2 Transfection of COS-1 cells with complexes made at a 1:2 (-/+) ratio in the presence and absence of fetal bovine serum. The transfection levels were compared with the transfection achieved using 2.5 μg plasmid/Lipofectamine (Lf) (1:6 w/w). Source: From Ref. [50].

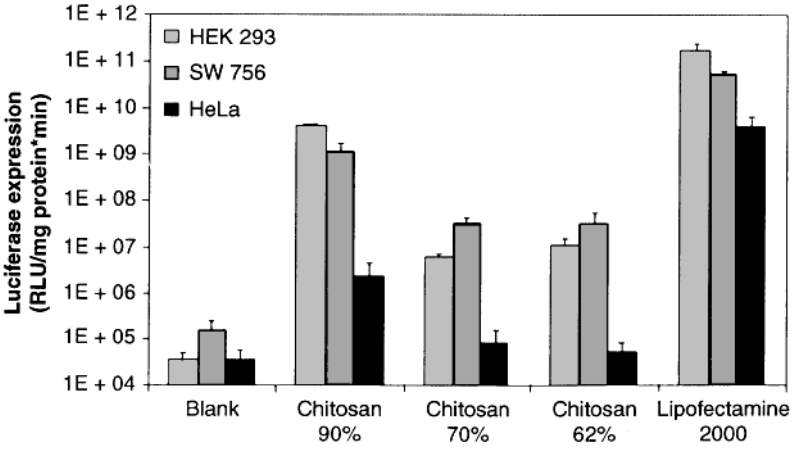


Figure 20.3 In vitro luciferase expression in all cell types tested with high molecular weight (390 kDa) chitosan–DNA nanoparticles. Gene expression was measured 3 days after transfection. Mean ± S.D. shown. Source: From Ref. [53].

Ishii *et al.* [64] explained the transfection mechanism of chitosan/DNA complexes in relation to cell uptake. They used fluorescein isothiocyanate-labeled plasmid DNA and Texas Red-labeled chitosan. They found that the transfection of chitosan/DNA complexes was higher with the molecular weight of chitosan at 40 or 84 kDa and N/P ratio at 5 (Figure 20.4). The transfection

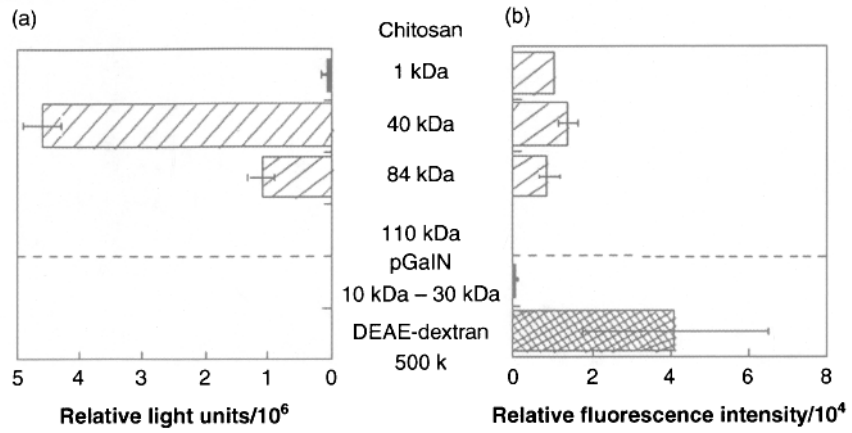


Figure 20.4 Effect of various gene transfer reagents on luciferase activity (a) and cell uptake (b) for SOJ cells. Transfection using plasmid/cationic polymer complexes at N/P = 5 was made in the presence of 10% FBS at a plasmid concentration of 1 μg/ml. Source: From Ref. [64].

medium contained 10% serum and the pH of the medium was 7. To explain the transfection mechanism, they investigated three different processes, such as (i) cell uptake, (ii) release from endosomes, and (iii) nuclear transport. They stated that during the transfection, the chitosan/DNA complexes condensed to form large aggregates, which then absorbed to the cell surface. After that, the complexes were endocytosed and released from endosomes. Then, they were released due to the swelling of lysosomes along with the swelling of the chitosan/DNA complex, causing the rupture of the endosome. Finally, the chitosan/DNA complexes were found to be accumulated in the nucleus.

However, low solubility, non-specificity, and low transfection efficiency of chitosan limited its clinical trials. Hence, chemical modification of chitosan became necessary to overcome its drawbacks for the clinical trials.

20.3.1.1 Modified Chitosans for Gene Therapy

20.3.1.1.1 Alkylated Chitosan

Like other polycations/DNA complexes, chitosan also forms complexes with DNA by electrostatic interaction, but the interaction is strong enough to resist DNA unpacking within the cell. Okano *et al.* [65], Sato *et al.* [66], and Kabanov *et al.* [67] reported that the transfection efficiency of chitosan could considerably increase by the incorporation of hydrophobic moieties into the chain backbone of chitosan. Liu *et al.* [68] investigated chitosan and alkylated chitosan vectors for gene transfection and the effects of hydrophobicity of the side alkyl chain on transfection activity. They prepared four types of alkylated chitosans having 4, 8, 12, and 16 carbon atoms in the alkyl chains. The transfection efficiency of chitosan and alkylated chitosans was observed in C_2C_{12} cell lines in the presence of fetal bovine serum (FBS). Although the size of the alkylated chitosan/DNA complexes was larger, the transfection efficiency of alkylated chitosan was increased many-fold compared to that of chitosan and DNA alone (Figure 20.5). They

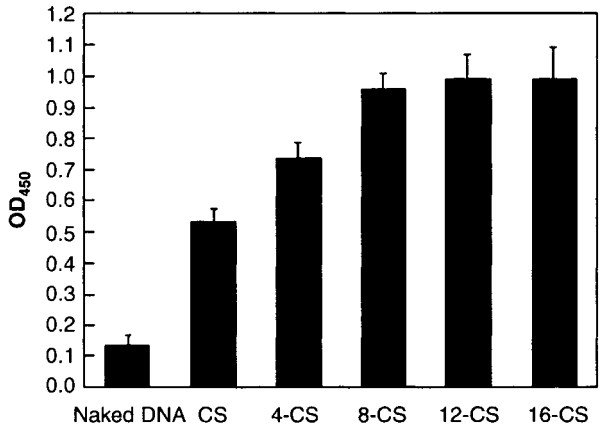


Figure 20.5 Transfection efficiency of pc DNA 3.1 plasmid encoding chloramphenicol acetyltransferase (CAT) mediated by chitosan (CS) and alkylated chitosan (ACS). Source: From Ref. [68].

were reported that the alkylated chitosan/DNA complexes transfected into the cell mainly via endocytosis because the destabilization of the membrane grew due to the increase in hydrophobicity of chitosan derivatives, which facilitated DNA entry into the cell. The incorporation of hydrophobic alkyl groups into the chitosan chain backbone not only increased the transfection efficiency but also increased the unpacking of DNA in the nucleus.

20.3.1.1.2 Quaternization of Oligomeric Chitosan

Thanou *et al.* [69] reported another technique to improve the transfection efficiency of chitosan by quaternization of oligomeric chitosan. They prepared quaternized chitosan oligomers and observed their transfection efficiency into COS-1 cells and Caco-2 cells. They synthesized N-trimethylated chitosan oligomers (TMO) by reductive methylation and the synthesized derivatives showed excellent solubility in water at different pH values. The TMOs formed smaller complexes with DNA at both 2:100 and 2:10 DNA/oligomers ratio compared to unmodified chitosan oligomers. They tested with TMO-40 (40% degree of quaternization) and TMO-50 (50% degree of quaternization) to observe the transfection efficiency into COS-1 and Caco-2 cells. They found that the transfection efficiencies of TMO-50 increased 5-fold (complexes with 1:6 ratio of DNA:oligomer) to 52-fold (complexes with 1:14 ratio of DNA:oligomer) compared to that of DNA alone (control group). But, TMO-40 showed higher transfection efficiencies ranging from 26-fold (ratio of DNA:oligomer was 1:6) to 131-fold (ratio of DNA:oligomer was 1:14) (Figure 20.6). They also found that the transfection efficiencies of quaternized oligomers was substantially lower in Caco-2 cells compared to the levels observed in COS-1 cells (Figure 20.7).

20.3.1.1.3 Urocanic Acid-modified Chitosan

In another study, Kim *et al.* [70] synthesized urocanic acid modified chitosan (UAC) to enhance the transfection efficiency. They prepared UAC by

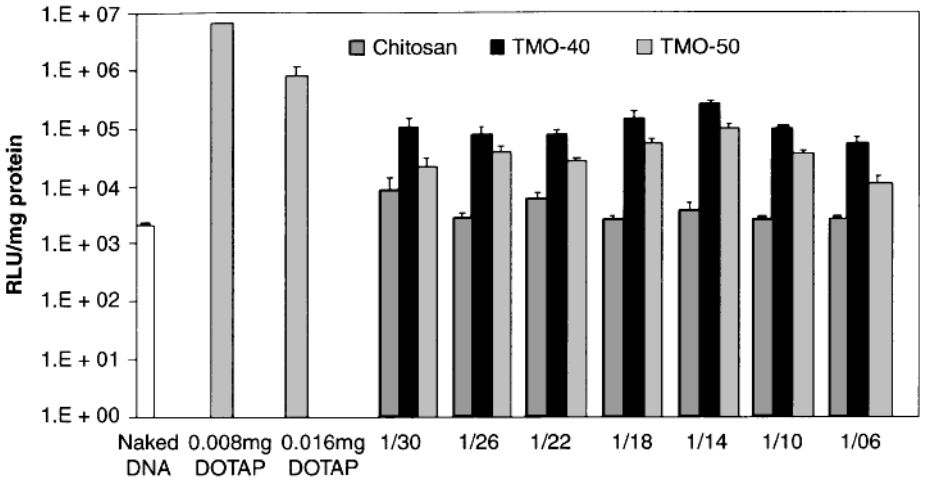


Figure 20.6 Transfection efficiencies in COS-1 cells obtained with chitosan oligomer, trimethylated chitosan oligomers and DOTAP (N-[1-(2,3-dioleoyloxy)propyl]-N,N, N-trimethylammonium sulphate)/DNA complexes at different weight/weight ratios. Values are mean ± S.D (n=3). Source: From Ref. [69].

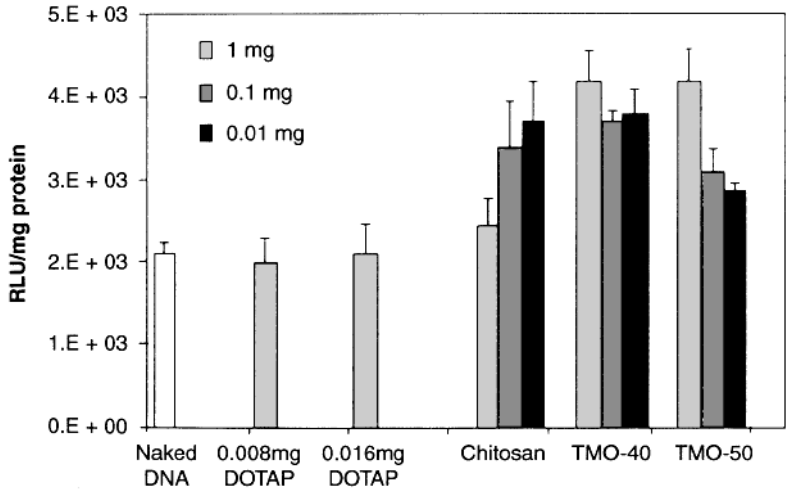
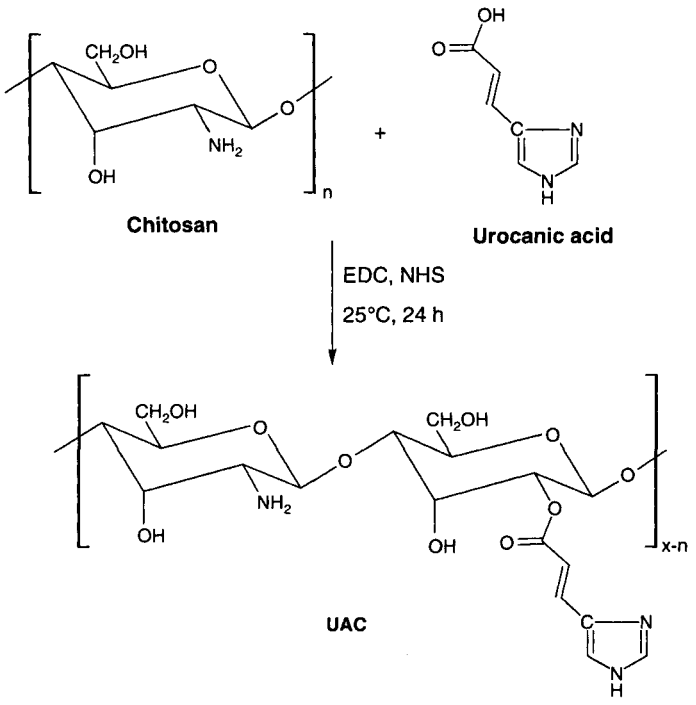


Figure 20.7 Transfection efficiencies in Caco-2 cells obtained with chitosan oligomer, trimethylated chitosan oligomers and DOTAP at different weight/weight ratios. Source: From Ref. [69].

conjugation of urocanic acid to chitosan using 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) mediated condensation (Scheme 20.2). They prepared UAC/DNA complexes by the mixing of three types of UAC, such as (i) UAC20 (20% degree of substitution of chitosan by urocanic acid), (ii) UAC50 (50% degree of substitution), and (iii) UAC70 (70% degree of substitution). It was found that all the derivatives complexed effectively with DNA at a higher N/P ratio (Figure 20.8). They obtained UAC/DNA complexes with an average diameter under 100 nm at all



Scheme 20.2 Synthetic scheme of UAC. Source: From Ref. [70].

charge ratios, while the amounts of aggregated complexes over 100 nm were negligible. The transfection of various UAC/DNA complexes was carried out into the 293T cells with different N/P ratios of the complexes. It was found that the transfection efficiency increases many folds with increasing the N/P ratios of the complexes compared to that of DNA alone (Figure 20.9). The transfection efficiency of UAC20 was almost the same as that of DNA alone below the N/P ratio of 5. This indicates that the proton sponge effect is inefficient due to the lack of UA in chitosan at below the N/P ratio of 5. They also transfected the UAC/DNA complexes into other cell lines, like HeLa cells (human cervix epithelial carcinoma cells), MCF-7 cells (breast adenocarcinoma cells), and NCTC 3749 cells (macrophase cell lines). They observed slightly enhanced transfection efficiency in the NCTC 3749 cell lines, but the enhanced transfection efficiency was not observed for the HeLa and MCF-7 cell lines, indicating the cell dependency of transfection. Corsi *et al.* [71] and Leong *et al.* [72] have also reported on the cell dependency of transfection.

20.3.1.1.4 Ethylenediaminetetraacetic Acid (EDTA) Conjugated Chitosan Loretz *et al.* [73] prepared chitosan-EDTA conjugate (CE) as a promising nonviral vector for gene transfer. They synthesized chitosan-EDTA conjugates by reaction of acidic chitosan solution with EDTA [with different weight ratios ranging from 1:40–1:10 of chitosan:EDTA] in the presence of EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide]. For the nanoparticle formation of CE

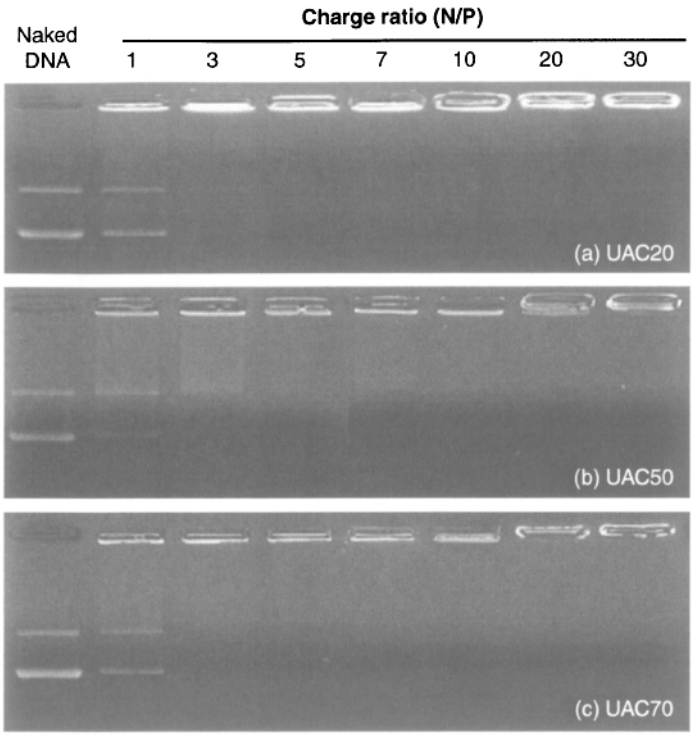


Figure 20.8 Analysis of complex formation of UAC/DNA (pEGFP N1: 4.7 kb) at the various charge ratios by agarose gel electrophoresis using 1% agarose in Tris-acetate running buffer. Panel (a): UAC15; panel (b) UAC50; panel (c): UAC70. Lane 1: plasmid DNA alone (0.2 μg) and lanes 2 through 8: DNA (0.2 μg) with progressively increasing proportions of UAC. Source: From Ref. [70].

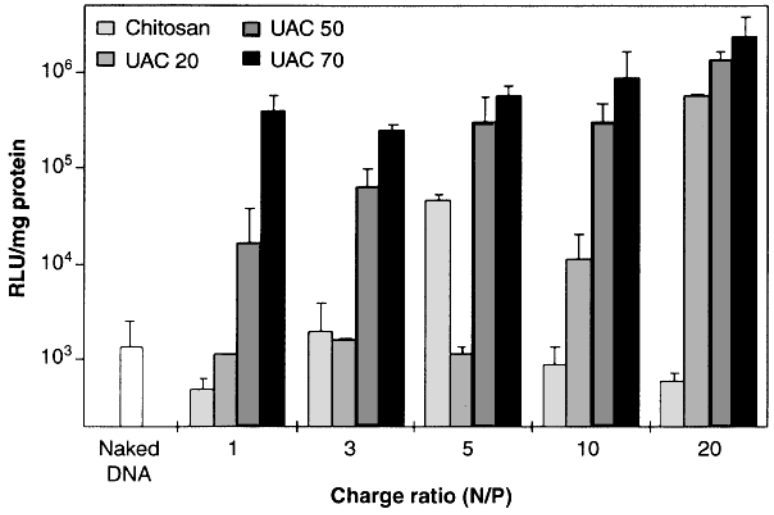


Figure 20.9 Transfection of 293T cells by UAC/DNA (pGL3-control) complexes prepared at various charge ratios. 293T cells were seeded at a density of $1x10^5$ cells/well in 24-well plate and incubated for 28 h before the addition of the polymer/DNA complexes. Transfection was performed at a dose of 1 μg of DNA for all groups and analyzed at 48 h after transfection (n = 3). Source: From Ref. [70].

with DNA, a weight ratio 1:12 instead of 1:2.5 of chitosan:EDTA was used. The size of the CE/DNA complexes were of nanoscaled size in the range of 27–88 nm. They transfected the CE/DNA nanoparticles against Caco-2 cells and the transfection efficiency of the nanoparticles was calculated from beta-galactosidase assay. The transfection efficiency was calculated by subtraction of the measured background activity of untransfected cells from the measured beta-galactosidase activity. From their study, it was found that CE/DNA complexes showed a considerably higher level of gene expression compared to that of chitosan and DNA alone, although, Caco-2 cell line is comparatively hard to transfect.

20.3.1.1.5 Deoxycholic Acid-modified Chitosan

To improve the transfection efficiency of chitosan, Kim *et al.* [74] modified the chitosan with deoxycholic acid. To prepare the deoxycholic acid modified chitosan (DAMC), firstly they depolymerized the chitosan into different molecular weights, such as 5 kDa, 25 kDa, 40 kDa, and 200 kDa, and the depolymerized chitosan was then hydrophobically modified with deoxycholic acid. After that, they prepared DAMC self-aggregates by sonication. The sizes of the DAMC self-aggregates were from 132 nm–300 nm. They found that the DAMC self-aggregates were fairly complexed with DNA. This was confirmed from the gradual decreasing of electrophoretic mobility of DNA on the agarose gel with increasing molecular weight of DMAC at the same N/P ratio (Figure 20.10). They studied the transfection efficiency of DAMC self-aggregates on COS-1 cell lines. From their study, it was found that the transfection efficiency for DMAC40k was the highest to deliver DNA into COS-1 cells, whereas DAMC5k self-aggregates showed the lowest efficiency in absence of serum (Figure 20.11). On the other hand, DAMC200k self-aggregates showed the highest transfection efficiency into the COS-1 cell lines compared to that of other self-aggregates as well as naked DNA in the presence of 80% serum. In the absence of serum, DAMC200k self-aggregates showed comparatively lower transfection efficiency than DAMC40k self-aggregates because they could form complexes with DNA so strongly that they would not release the DNA easily into the cells. In the presence of serum, the abundant proteins of serum inhibit the complex formation by weakening the ionic interaction

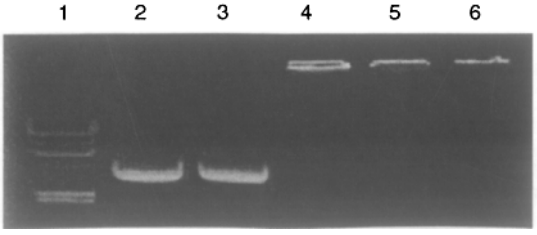


Figure 20.10 Electrophoresis of DAMC self-aggregate/DNA complexes on an agarose gel. Lane 1, DNA molecular weight marker; lane 2, DNA only; lane 3, DAMC5k/DNA complex; lane 4, DAMC25k/DNA complex; lane 5, DAMC40k/DNA complex; lane 6, DAMC150k/DNA complex at the charge ratio (±) of 4/1. Source: From Ref. [74].

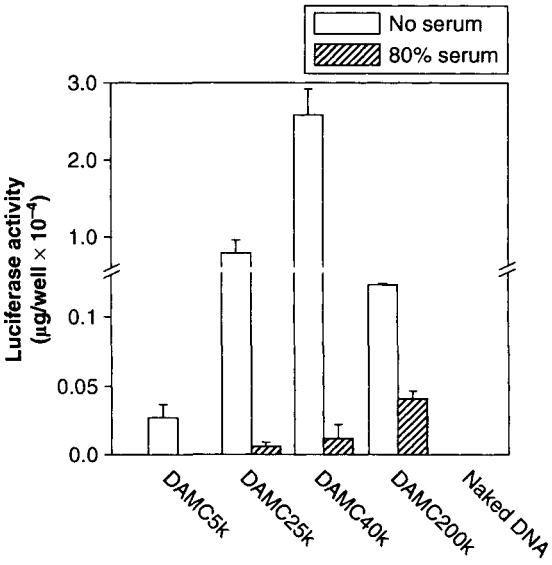


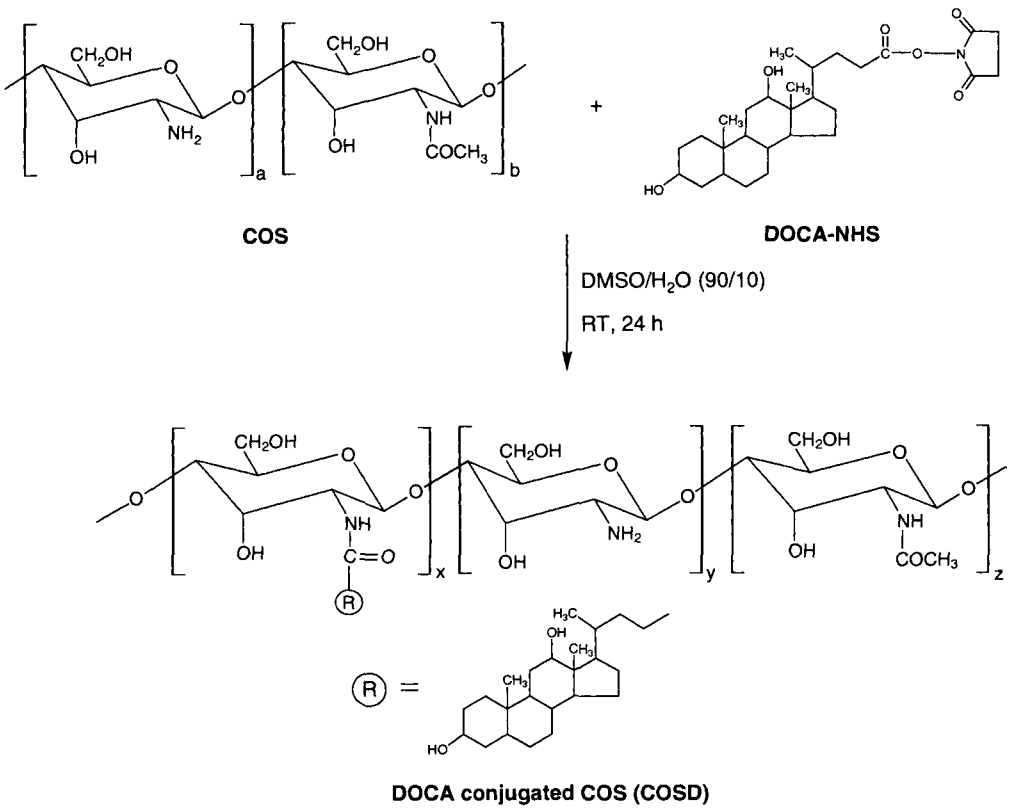
Figure 20.11 In vitro transfection activity of DAMC self-aggregate/pCMV-Luc+ complexes in COS-1 cells. Gene expression was determined by luciferase activity using a Promega kit. Source: From Ref. [74].

between DAMC self-aggregates and DNA, resulting in an increase in the transfection efficiency of DAMC200k self-aggregates.

In another study, S. Y. Chae *et al.* [75] prepared deoxycholic acid modified chitosan oligosaccharide (COS) as effective gene carrier. They used chitosan oligosaccharide to improve the solubility of chitosan, to reduce toxicity, and to increase the DNA unpacking after cellular uptake of polymer/DNA complexes. They synthesized the conjugates by coupling reaction of NHS-ester of deoxycholic acid and chitosan oligosaccharide as shown in Scheme 20.3. The sizes of the chitosan oligosaccharide deoxycholic acid conjugate (COSD)/DNA complexes were reported in the range of 80–200 nm. It was also found that COS/DNA and COSD/DNA complexes showed no toxicity effect on the cells, and the relative cell viabilities were maintained around 100% (Figure 20.12). They studied the transfection efficiency of COSD into HEK 293 cells using pEGFP-N1 plasmid encoding green fluorescence protein (GFP). They found that the transfection efficiency of COSD/DNA complexes increased manyfold compared to that of naked DNA, PLL, and COS3 (molecular weight 1-3 kDa). The transfection efficiency of COS3D25 (25% degree of substitution by deoxycholic acid) increased more than 1000 times than that of COS3 with carrier/DNA weight ratio of 30, and they also found that the transfection efficiency decreased with an increasing degree of substitution (Figure 20.13).

20.3.1.1.6 Galactosylated Chitosan

To improve the transfection efficiency and cell specificity, Gao *et al.* [76] depolymerized the high molecular weight chitosan (HMWC) into low molecular weight chitosan (LMWC), and then it was modified with galactose group. They



Scheme 20.3 Synthetic schemes for DOCA conjugation on COSs. Source: From Ref. [75].

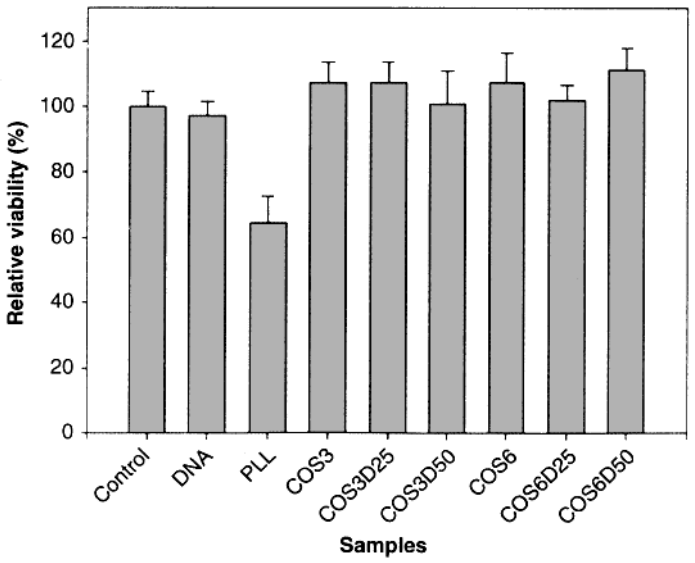


Figure 20.12 Cytotoxicity of Carrier/plasmid DNA complexes on HEK 293 cells. The chitosan based complexes were prepared by carrier /DNA weight ratio of 30 (+/_) and PLL/DNA complex was prepared by the ratio of 2. The cells were treated with complex for 4 h and the MTT assay was performed at 24 h after the complex treatment. Source: From Ref. [75].

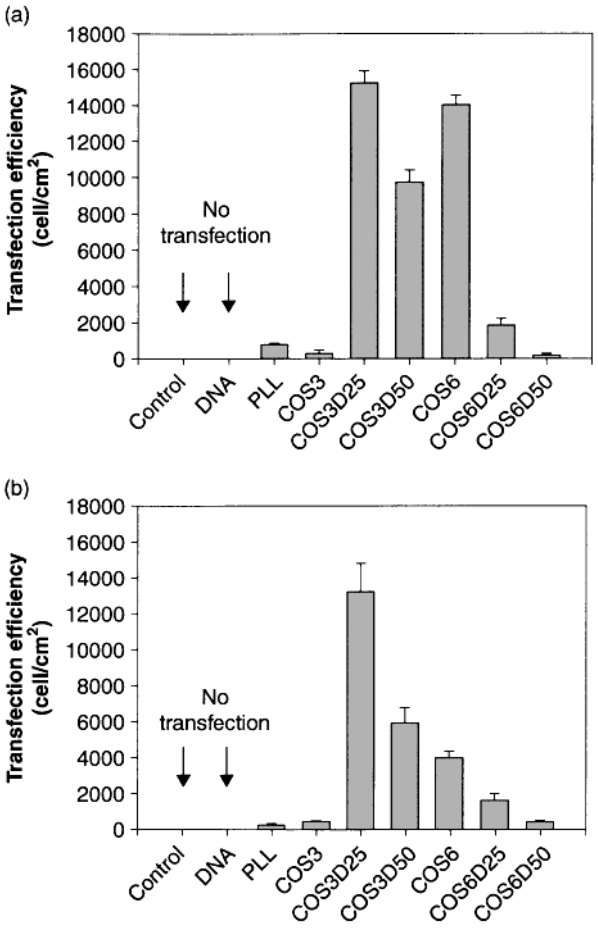
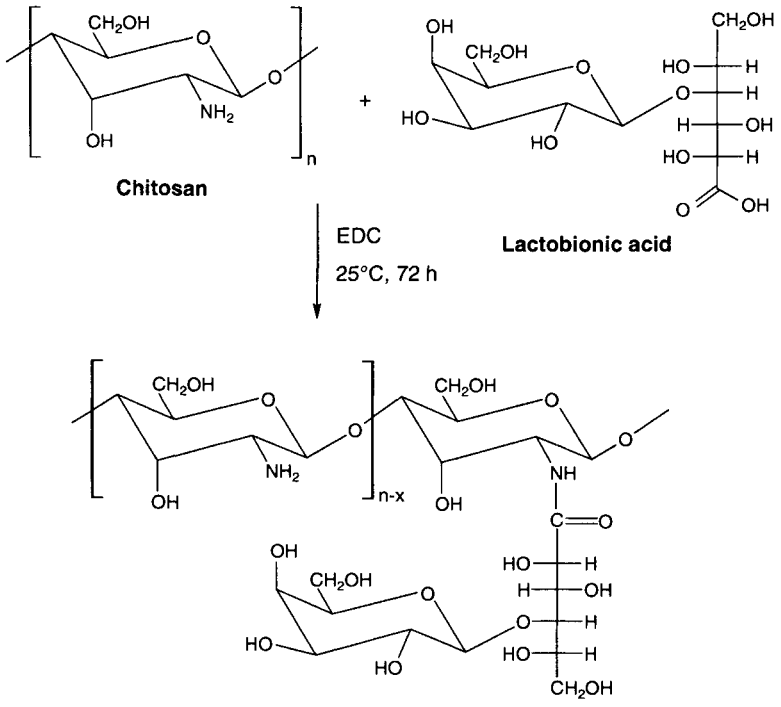


Figure 20.13 The carrier dependent transfection efficiencies measured at media pH 6.5. The HEK 293 cells were incubated with carrier/plasmid DNA complexes for 4 h (with and without FBA) and the transfection efficiencies were observed at 72 h after the transfection. (A) The transfection efficiencies were evaluated by the transfected cellcounting (without FBS). (B) The gene transfections in the presence of 10% FBS. The carrier /DNA weight ratio of COS based carriers was 30 (+/_) and PLL/DNA complex was prepared by the ratio of 2. Data represent mean ±standard deviations (n = 4). Source: From Ref. [75].

depolymerized the HMWC with hydrochloric acid. Then, the obtained LMWC was coupled with lactobionic acid (LA) in the presence of EDC (Scheme 20.4). Chitosan/DNA complexes were prepared by coacervation process. The sizes of LMWC/DNA complexes were approximately 220 nm, whereas the sizes of galactosylated LMWC (gal-LMWC)/DNA complexes were around 350 nm. They examined the transfection efficiency of gal-LMWC into HepG2 cells. It was found that the β-galactosidase activity increases with an increase in the N/P ratio. But, the β-galactosidase activity reached maxima at N/P ratio of 5.6, beyond which it declined (Figure 20.14). At N/P ratio of 5.6, the gal-LMWC was complexed completely with DNA and entered into the cell through receptor-mediated endocytosis pathway. Above a N/P ratio of 5.6, the activity



Scheme 20.4 Synthesis of galactosylated chitosan. Source: From Ref. [76].

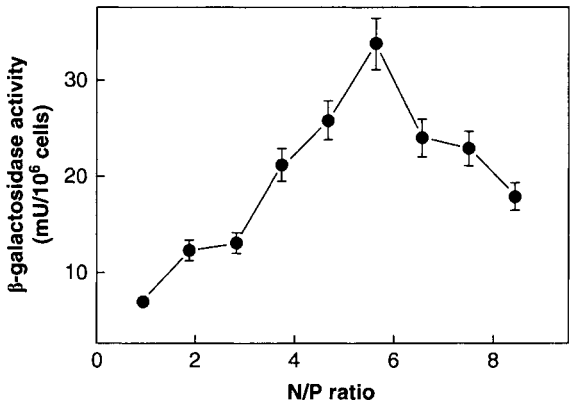


Figure 20.14 Charge-dependent β-galactosidase expression in HepG2 cells transfected with gal-LMWC/DNA complexes. Results are expressed as mean values ± S.D. from one representative experiment ($n = 4$) of three performed ($P < 0.05$). Source: From Ref. [76].

decreased. There were many free gal-LMWC, which combined with receptors on the cell surface competitively and inhibited the coupling of gal-LMWC/DNA complexes with the receptors. This caused a reduction in the transfection efficiency of the complexes by inhibiting the endocytosis process. From this study, it was also found that the transfection efficiency of gal-LMWC/DNA complex was almost the same as that of naked DNA in the HeLa cells without asialoglycoprotein receptor (ASGR) on the cell membrane surface. But, the

gal-LMWC/DNA complex had the capability to transfect selectively the HeLa cell lines with ASGR.

20.3.2 Gelatin

Gelatin has been widely used as a carrier of protein because of its ability in lowering protein degradation capacity [77, 78]. Gelatin has extensively been used in industrial, pharmaceutical, and medical applications because the physicochemical nature of gelatin can easily be changed by chemical modification. For example, positively charged cationic gelatin can readily be prepared by introducing amine residues to the carboxyl groups of gelatin. Therefore, researchers are stimulated by these advantages of gelatin to develop a new gene delivery system using cationized gelatin hydrogel as a carrier.

Gelatin is obtained by thermal denaturation of collagen, which is also isolated from animal skin and bones. In its structural unit, it contains many glycine units, proline and 4-hydroxyproline residues.

Gelatin shows excellent controlled biodegradation of local delivery agent and it can also protect plasmid DNA from rapid degradation by nucleases [79–81]. Gelatin derivatives, obtained by chemical introduction of different amine compounds, such as ethylenediamine (Ed), spermidine (Sd), and spermine (Sm) are also used as plasmid DNA carrier. Among the amine derivatives of gelatin, the Sm derivative is the most effective gene carrier due to its superior buffering ability compared to the Ed and Sd derivatives [82].

Hosseinkhani *et al.* [82] prepared amine derivatives of gelatin with different extents of amine contents. They synthesized gelatin-amine derivatives by introduction of Ed, Sd, and Sm into the carboxyl groups of gelatin with the help of the conventional EDC method [82]. They observed that the amine derivatives of gelatin were complexed effectively with DNA compared to that of gelatin alone, and they also obtained smaller particle (below 150 nm) with amine derivatives compared to gelatin. They transfected the gelatin derivatives into rat gastric mucosal (RGM)-1 cells by applying each with ultrasound irradiation. From their study, it was found that the transfection efficiency was significantly increased by the gelatin derivative compared to that of DNA alone and gelatin. Sm derivative complex showed higher transfection efficiency than that of the corresponding other complexes. It was also observed that ultrasound irradiation increased the transfection efficiency of every complex, but the enhancement was saturated at the complex to DNA ratio of 5.0.

Recently S. Kommareddy and M. Amiji [83] studied the potential of engineered gelatin-based nanoparticulate vectors for systemic delivery of therapeutic genes to human solid tumor xenografts *in vivo*. In this study, they synthesized thiolated gelatin by covalent modification of the epsilon-amino groups gelatin with 2-iminothiolane to enhance the intracellular delivery potential of the gelatin. They also modified the surface of both gelatin and thiolated gelatin nanoparticles by reacting with methoxy-poly(ethylene glycol) (PEG)-succinimidyl glutarate to prolong *in vivo* circulation time, enabling the medication to stay in the body for up to 15 hours. This had been only

three hours with unmodified nanoparticles. The modification of the gelatin with PEG also enhanced tumor uptake and retention of the nanoparticles after administration. They obtained highest levels of protein (sFlt-1 or soluble receptor for an angiogenic factor, that cut off blood supply to the tumor) expression with PEG-modified gelatin nanoparticles *in vitro* in MDA-MB-435 human breast adenocarcinoma cell line.

In another study, Wang *et al.* [84] prepared gelatin-siloxane nanoparticles (GS NPs) and then coupled them with HIV-1 Tat peptide with the GS surface via sulfhydryl groups to achieve efficient gene transfection and expression. They synthesized the Tat peptide decorated gelatin-siloxane nanoparticles (TG NPs) in three steps. Firstly, gelatin-siloxane nanoparticles were prepared by a two-step sol-gel procedure, secondly, sulfhydryl (-SH) groups were introduced on the GS NPs, and finally SH-GS NPs were coupled with synthetic Tat peptide. They observed that TG NPs were nontoxic at concentrations <300 μg ml^{-1} towards the HeLa cells. They studied the cellular uptake of fluorescein iso-thiocyanate (FITC)-labeled TG NPs into HeLa cells. They found more TG NPs in the intracellular region than GS NPs, which demonstrated that the cellular uptake of nanoparticles might be promoted by Tat peptide. They also observed that the cellular uptake of FITC-labeled TG NPs was increased with an increase in incubation temperature from 4°C–37°C, which indicated that the cellular uptake of TG NPs occurred through an internalization endocytosis mechanism, because the endocytosis is an energy dependent process. The transfection efficiency of TG/DNA nanocomplexes were examined in HeLa cells by using pSV β-galactosidase assay. It was found that the transfection efficiency increased about 1.3 fold with TG/DNA (100:1 g g^{-1}) and Tat/GS (1.58 μmol g^{-1}) compared to the commercially available gene carrier Lipofectamine. But, the transfection efficiency of TG/DNA <50:1 (g g^{-1}) was limited due to the formation of large complex (>0.8 μm). They also investigated the effect of serum on the transfection efficiency of the TG/DNA nanocomplexes and found that the transfection efficiency of TG/DNA nanocomplexes was dramatically decreased in the presence of serum.

20.3.3 Alginate

Alginate is a naturally occurring linear polysaccharide that is abundantly produced by brown algae, and by bacterial species of Pseudomonas [85] and Azotobacter [86]. It is composed of unbranched β-1,4 linked D-mannuronic acid (M) and variable amounts of its C5-epimer, L-guluronic acid (G). Many divalent cations, like Ca^{2+}, Ba^{2+}, Sr^{2+}, and more, with the exception of Mg^{2+} are used to form alginate hydrogel. The hydrogel forming character of alginate makes it a very attractive choice as a matrix for the encapsulation of biological, including drug-containing liposomes and cells. Alginate encapsulated liposomes have been studied for protein delivery [87–89] and genetically engineered fibroblasts have been encapsulated in alginate for therapeutic applications [90, 91].

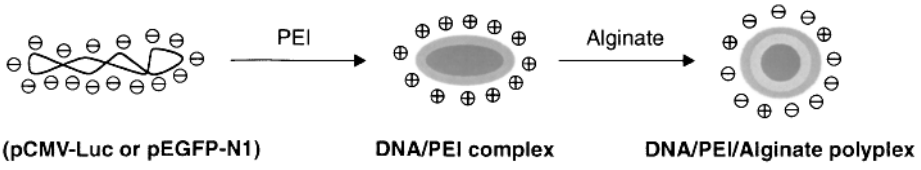
It is reported that polyethylenimine (PEI) is an efficient gene delivery vehicle due to its better stability, easy handling, and lower cost compared to

cationic liposomes [92]. Although it can condense DNA effectively and exhibits endosomolytic activity at acidic endosomal pHs, which makes it efficient nonviral vectors, the transfection efficiency of PEI is low in the presence of serum [93, 94]. To improve the serum stability and lower toxicity with higher transfection efficiency of PEI, negatively charged sodium alginate was used to coat the positively charged PEI/DNA complex [95, 96]. It has also been reported that the trans-gene expression can be enhanced by encapsulating the DNA with alginate and the immune response can also be mitigated by the alginate delivery system [96].

Jiang *et al.* [97] prepared a novel nonviral system, DNA/PEI/Alginate (DPA) polyplex for the delivery of DNA, and they observed the delivery both *in vitro* and *in vivo*. To prepare this novel nonviral delivery system, they first prepared DNA/PEI (DP) complex and then the DP complex was coated by alginate (Scheme 20.5). They also coated the DP complex with poly(methacrylic acid) (PMA) and poly(acrylic acid) to compare with alginate coated DP complex. They carried out the *in vitro* gene transfection tests by using C3 cells and the *in vivo* gene transfection tests were carried out in a six week old female C57/BL6 mouse. They observed that the particle size of the DPA complex decreased slightly and then increased by increasing the alginate content, and the zeta potential decreased due to the negative charge of the alginate. They also noticed that the cytotoxicity of the complex markedly decreased by the alginate coating. It was found that the luciferase gene transfection efficiency in C3 cells increased 10–30 fold with the DPA complex compared to that of DP complex in the presence of 50 vol% serum. Molecular weight of alginate also affected the luciferase gene transfection and it was shown that low molecular weight alginate had highest gene transfection efficiency. To investigate *in vivo* gene transfection, they firstly produced C3 cell-induced model tumor into C57/BL6 mice and then either DP or DPA polyplex with a weight ratio of alginate to DNA of 0.15 was injected into the model tumor. It was found that DPA polyplex showed approximately a 7-fold higher luciferase gene expression compared to that of DP complex (Figure 20.15).

20.3.4 Arginine

In recent times, it is known that peptides such as Tat (48-60), Antp (43-58), and VP22 (267-300), containing highly basic amino acids (arginine and lysine), have been widely used as cellular delivery vectors [98–100]. It is also known



Scheme 20.5 Schematic representations of the preparation of the DNA/PEI complex and the following DNA/PEI/Alginate polyplex. Source: From Ref. [97].

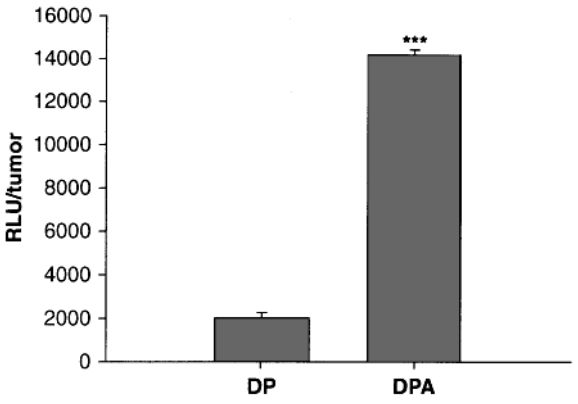


Figure 20.15 Transfection efficiency of the DNA/PEI (DP) complex or the DNA/PEI/Alginate (DPA) polyplex in C57/BL6 mouse tumor models (n = 3) prepared by the subcutaneous injection of C3 cells: luciferase gene (pCMV-Luc) expression level in C3 tumors treated with DP and DPA polyplex (mean ± SD, n = 3); *** significantly different from DP by t-test (p = <0.001). Source: From Ref. [97].

that peptides composed of only arginine residues are able to translocate efficiently through the cell membrane [101].

Arginine is an α-amino acid and is one of the twenty most common natural amino acids [102]. The side chain of arginine consists of a 3-carbon aliphatic straight chain with a complex guanidinium group at the end (Figure 20.16). It is synthesized mainly from citrulline. Citrulline can also be obtained from many sources such as, from arginine via nitric oxide synthase (NOS), from ornithine via catabolism of prolone or glutamine/glutamate, and from asymmetric dimethylarginine.

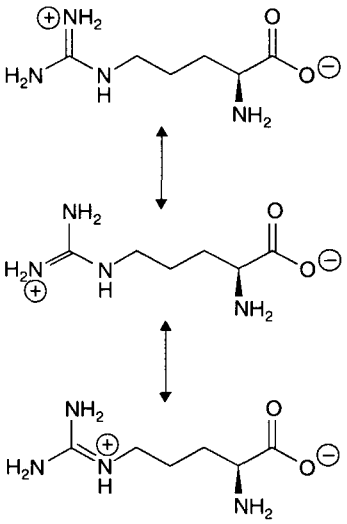


Figure 20.16 Delocalization of charge in guanidinium group of L-Arginine.

Choi *et al.* [103] observed the gene delivery mechanism of the arginine peptide system. They investigated the effect of the arginine/DNA complex size on the transfection efficiency. They synthesized arginine peptides (R15) by solid phase peptide synthesis using standard 9-fluorenylmethyloxyccarbonyl (FOMC) chemistry. They used rhodamine labeled DNA and FITC tagged peptides to investigate the structure of arginine peptide/DNA complexes. They found that the size of the complexes increased with an increase in the incubation time in serum free medium. The mean diameter of the complexes was 400 nm at the time of immediate formation of complexes. But after 1hr of incubation, the size of complexes increased to approximately 6 μm. Large sized complexes of up to 26 μm were obtained after 2 hr incubation. Previous studies [104–106] reported that the formation of larger structure with higher incubation time was probably due to the aggregation of small complexes. They observed the cellular uptake and transfection efficiency of arginine peptide/DNA complexes on the 293T cells. It was found that there was no relationship between the complex size and the cellular distribution of the complex. The large complexes, 6 μm and 26 μm, showed a similar degree of cellular uptake as that of 400 nm sized complex. The FACS (fluorescence-activated cell sorting) analysis showed that small-sized complex (400 nm) had only 20% higher cellular uptake compared to the large complexes.

They also investigated the localization of arginine peptide/DNA complexes in the nucleus. Earlier studies showed that there was a major barrier in nuclear transportation for nonviral gene transfer [107, 108]. They used double labeled complexes with rhodamine and FITC for the determination of the nuclear transport of arginine peptide/DNA complexes. They observed the fluorescence predominantly on the extracellular surface after 1hr incubation and after 2 hr incubation, the fluorescence was found in the cytoplasm, which indicated the entry of the complexes into the cells. After 6 hr incubation, they observed the fluorescence in a perinuclear location and finally, the fluorescence was observed within the nucleus after 12 hr incubation. From this observation, they concluded that the arginine peptide/DNA complexes were transferred into the cell nucleus without the dissociation of the arginine peptide.

Rossenberg *et al.* [109] prepared arginine containing oligopeptide stable polyplex as nonviral gene delivery vector. They designed a series of peptide analogs in which arginine moieties increased by partial replacing of lysine residues. The stability of polyplex was enhanced by increasing the cationic charge of the arginine peptide. They found that arginine-containing peptides (GM102 and GM202) complexed effectively with DNA at a peptide to DNA charge ratio of 1.5 and formed comparatively smaller complex of approximately 20 nm in size. They observed the transfection efficiency of the various polyplexes into HepG2 cells. The transfection efficiency increased by 5-fold with the arginine containing peptides/DNA complexes as compared to naked DNA.

20.3.5 Collagen

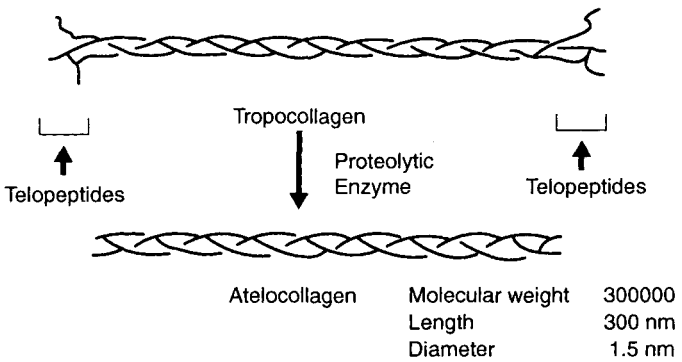
For many years, collagen has been used in medical applications, like resorbable surgical sutures [110], hemostatic agents [111], and wound

dressings [112], due to its excellent biocompatibility [113] and many unique characteristics [114].

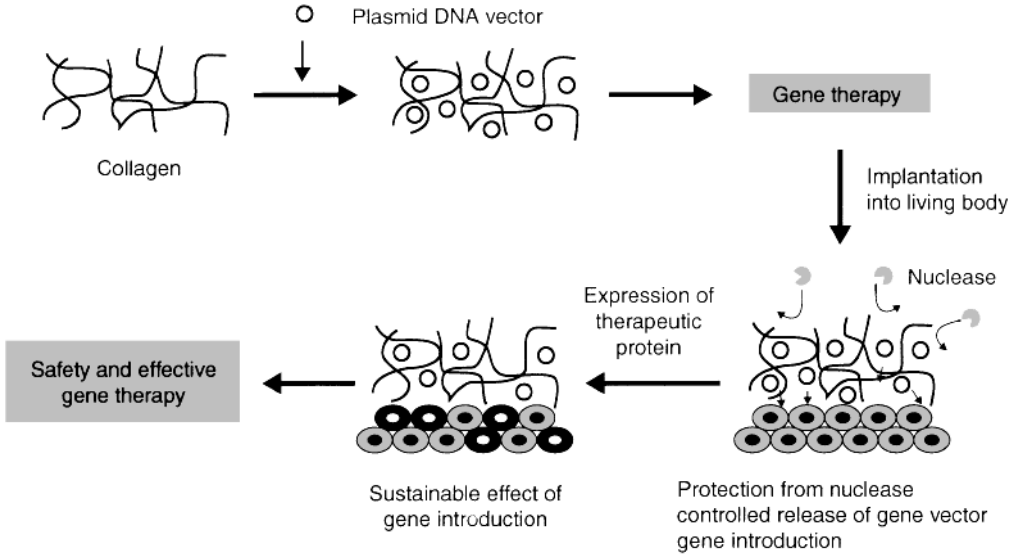
Collagen is the main protein of connective tissue in animals and is fibrous in nature. It connects and supports other bodily tissues, such as skin, bone, tendons, muscles, and cartilage. It also supports the internal organs and is even present in teeth. In fact, it makes up about one third of the total amount of proteins in the body. Collagen is composed of three helical polypeptide chains with a rod-like structure and has a molecular weight of about 300,000 and a length of 300 nm. Atelocollagen may be obtained from collagen by removing the non-helical telopeptides attached to both ends of the collagen molecule, with pepsin treatment (Scheme 20.6). In the past years, atelocollagen gel has been widely used for the repair of skin depressions in plastic surgery and for subcutaneous injection [115]. Currently, atelocollagen is being investigated for a wide range of drug and gene delivery methods [116].

Collagen can be used as a carrier in the delivery of gene vectors and pDNA due to some of its outstanding properties, such as (i) it remains in liquid form at a low temperature and (ii) it exhibits a plasticity nature i.e. it becomes fibrous and then solid due to body temperature when it is implanted in the body. Therefore, gene vector and pDNA can be easily mixed with collagen to form collagen/pDNA or gene vector complex due to the above properties. Collagen turns into fibrous form from the liquid state after administration into the body, and then it forms a matrix structure which protects the gene vector or pDNA from immunological reaction and enzymatic attack (Scheme 20.7). Instead of the liquid form, gene vector or pDNA can also be transferred with collagen by means of beads, sponges, membranes, Minipellets, and various other forms.

Honma *et al.* [117] developed a novel technology for transfer of gene medicines including pDNA expression constructs, antisense ODNs and virus vectors in mammalian cells by atelocollagen mediated gene carrier. They prepared nanosized particles of atelocollagen/genetic material complexes and then the nanoparticles were precoated on a micro-well plate on which the cells were seeded. The size of the nanosized particles of atelocollagen/pDNA



Scheme 20.6 Schematic of collagen molecule. Source: From Ref. [116].



Scheme 20.7 Conception of collagen-mediated gene delivery. Source: From Ref. [116].

(8:10 ratio μg/ml) was in the range of 100–200 nm and showed maximal transfection efficiency in the 293 cells. They also studied the transfection efficiency of atelocollagen/oligodeoxynucleotide (ODN) complex into the NEC8 cells (human testicular tumor cells). They observed that the growth of tumor cells was inhibited by the ODN complexes, although it was in a ODN-dose dependent manner.

In another report, Wang *et al.* [118] prepared methylated collagen (MC) as gene carrier. MC was prepared by methylation of the carboxyl groups of collagen. Due to increased positive charge of MC compared to collagen, MC easily formed complexes with negatively charged DNA at neutral pH and also improved the stability of the complexes at the physiological conditions. MC condensed DNA more effectively even at pH 7.4 and the charge density of the complex was also higher than the native collagen at pH 3. The cytotoxicity of MC was lower than two widely used polymeric carriers, such as PLL and PEI. The transfection efficiency of MC/DNA complex against HEK293 cells increased about 18.9-fold compared with native collagen/DNA complex at a weight ratio of 5. This occurred due to the improved stability of DNA by MC. The transfection efficiency of the MC/DNA complexes decreased with increasing the weight ratio of MC/DNA. But, the transfection efficiency of MC/DNA complex further increased at higher weight ratios of 10 and 15 in the presence of chloroquine in the transfection medium. The same results were obtained for native collagen/DNA complexes, although the gene expression levels were much lower than MC/DNA complexes. The *in vivo* study resulted in higher luciferase expression (3.8-fold) by the native collagen/DNA complexes compared to that of naked DNA or MC/DNA complexes. The dissimilarity between the *in vitro* and *in vivo* results is yet unclear.

20.4 Conclusions

The application of gene therapy towards genetic diseases holds great promise. Although the preferred vectors in the field of gene therapy are viral vectors and synthetic polymeric vectors, natural polymers have unique and intrinsic properties that favour them to serve as a gene carrier. Natural polymers are generally nontoxic even in large doses because they are mucoadhesive, biocompatible, and biodegradable [119]. Chitosan is a naturally occurring linear binary cationic polysaccharide that is generally derived from shellfish. Chitosan has been successfully used as a nasal, ocular, and peroral drug delivery system that prolongs contact time as well as improves drug absorption [48]. Apart from this, chitosan also has been used as an oral and nasal gene delivery for vaccinations [120–122]. It was found that low transfection efficiency, low solubility, and the non-specificity of chitosan limits its use in clinical trials. Chemical modifications improved these drawbacks however. Although gelatin, alginate, arginine, and collagen systems show limited gene transfer success *in vivo*, modified forms of these systems have shown a capacity for the extended release of genes. Appropriate modifications of the natural forms of these polymers can make them targeted gene delivery systems to specific cell types, and can also improve their transfection efficiency, as well as extend their residence time once delivered *in vivo*. From the current research, it was found that the mechanism of intracellular escape and transfection by natural polymers occurs in different ways than that of synthetic polymers, including cell surface level, endosome release level, nuclear transport level, and any other potential rate-limiting steps [123–126]. The transfection efficiency of polymeric gene carrier can be improved by modifying some factors, such as the molecular weight of polymer, charge density of the polymer, overall surface charge of gene/polymeric vector, surface ligands as well as enhancement of availability near the cell surface [127–131]. Therefore, natural polymers can be efficiently used as nonviral vectors in gene therapy.

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