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Polylactic Acid: Synthesis, Properties and Applications

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ABSTRACT

Polylactic acid (PLA) is at present one of the most promising biodegradable polymers (biopolymers) and has been the subject of abundant literature over the last decade. PLA can be processed with a large number of techniques and is commercially available (large-scale production) in a wide range of grades. It is relatively cheap and has some remarkable properties, which make it suitable for different applications. This chapter deals with the different syntheses to produce this biopolymer, its diverse properties and various applications. Its biodegradability is adapted to short-term packaging, and its biocompatibility in contact with living tissues is exploited for biomedical applications (implants, sutures, drug encapsulation …).

Keywords

Polylactic acid, Biopolymer, Biodegradable, Properties, Synthesis, Process, Application, Packaging, Biomedical

21.1 INTRODUCTION

Tailoring new materials within a perspective of eco-design or sustainable development is a philosophy that is applied to more and more materials. It is the reason why material components such as biodegradable polymers can be considered as 'interesting' – environmentally safe – alternatives. Besides, ecological concerns have resulted in a resumed interest in renewable resources-based products.

Figure 21.1 shows an attempt to classify the biodegradable polymers into two groups and four different families. The main groups are (i) the agro-polymers (polysaccharides, proteins, etc.) and (ii) the biopolyesters (biodegradable polyesters) such as polylactic acid (PLA), polyhydroxyalkanoate (PHA), aromatic and aliphatic copolyesters [1]. Biodegradable polymers show a large range of properties and can now compete with non-biodegradable thermoplastics in different fields (packaging, textile, biomedical, etc.). Among these biopolyesters, PLA is at present one of the most promising biopolymer. PLA has been the subject of an abundant literature with several reviews and book chapters [2–8], mainly during the last decade. PLA can be processed with a large number of techniques. PLA is commercially and largely available (large-scale production) in a wide range of grades. It has a reasonable price and some remarkable properties to fulfil different applications. For instance, the PLA production capacity of Cargill (USA) in 2006 was 140 kT per year at 2–5 Euros per kg [9]. Other companies, such as Mitsui Chemical (Lacea-Japan), Treofan (Netherland), Galactic (Belgium), Shimadzu Corporation (Japan), produce smaller quantities. Some of them are only focused on the biomedical market like Boeringher Ingelheim (Germany), Purac (Netherland) or Phusis (France), because the constraints of this market are very specific. However, according to different sources, PLA consumption in 2006 was only about 60 000 tons per year and, at present, only ~30 per cent of lactic acid is used for PLA production. Thus, this biopolymer presents a high potential for development.

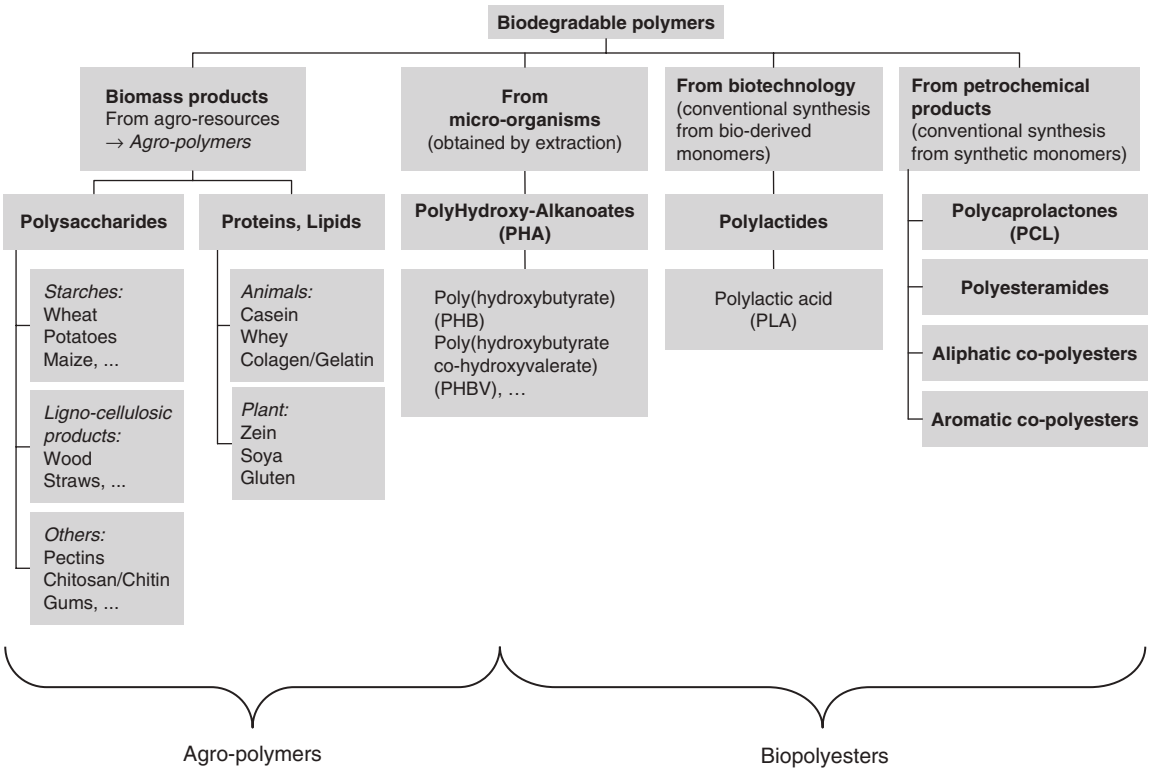

Figure 21.1 Classification of the biodegradable polymers. (Adapted from Reference [1].)

PLA belongs to the family of aliphatic polyesters commonly made from α-hydroxy acids, which also includes, for example, polyglycolic acid (PGA). It is one of the few polymers in which the stereochemical structure can easily be modified by polymerizing a controlled mixture ofland D isomers (Fig. 21.2) to yield high molecular weight and amorphous or semi-crystalline polymers. Properties can be both modified through the variation of isomers (l/d ratio) and the homo and (d,l)copolymers relative contents. Besides, PLA can be tailored by formulation involving adding plasticizers, other biopolymers, fillers, etc.

PLA is considered both as biodegradable (*e.g.* adapted for short-term packaging) and as biocompatible in contact with living tissues (*e.g.* for biomedical applications such as implants, sutures, drug encapsulation, etc.). PLA can be degraded by abiotic degradation (*i.e.* simple hydrolysis of the ester bond without requiring the presence of enzymes to catalyze it). During the biodegradation process, and only in a second step, the enzymes degrade the residual oligomers till final mineralization (biotic degradation).

As long as the basic monomers (lactic acid) are produced from renewable resources (carbohydrates) by fermentation, PLA complies with the rising worldwide concept of sustainable development and is classified as an environmentally friendly material.

21.2 SYNTHESIS OF PLA

The synthesis of PLA is a multistep process which starts from the production of lactic acid and ends with its polymerization [2–4, 6–7]. An intermediate step is often the formation of the lactide. Figure 21.2 shows that the synthesis of PLA can follow three main routes. Lactic acid is condensation polymerized to yield a low molecular weight, brittle polymer, which, for the most part, is unusable, unless external coupling agents are employed to increase its chains length. Second route is the azeotropic dehydrative condensation of lactic acid. It can yield high

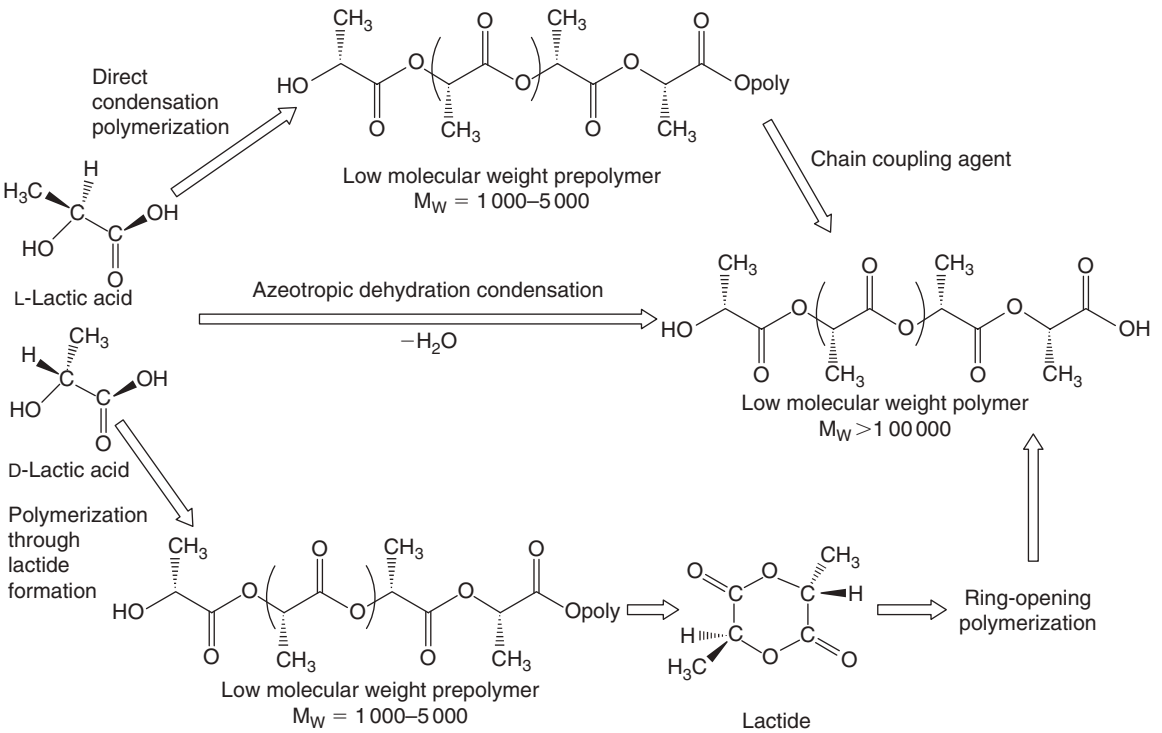

Figure 21.2 Synthesis methods for obtaining high molecular weight. (Adapted from Reference [3].)

molecular weight PLA without the use of chain extenders or special adjuvents [3]. The third and main process is ring-opening polymerization (ROP) of lactide to obtain high molecular weight PLA, patented by Cargill (US) in 1992 [8–9]. Finally, lactic acid units can be part of a more complex macromolecular architecture as in copolymers.

21.2.1 Precursors

21.2.1.1 Lactic acid

Lactic acid is a compound that plays a key role in several biochemical processes. For instance, lactate is constantly produced and eliminated during normal metabolism and physical exercise. Lactic acid has been produced on an industrial scale since the end of the nineteenth century and is mainly used in the food industry to act, for example, as an acidity regulator, but also in cosmetics, pharmaceuticals and animal feed. It is, additionally, the monomeric precursor of PLA. It can be obtained either by carbohydrate fermentation or by common chemical synthesis. Also known as 'milk acid', lactic acid is the simplest hydroxyl acid with an asymmetric carbon atom and two optically active configurations, namely the L and D isomers (Fig. 21.2), which can be produced in bacterial systems, whereas mammalian organisms only produce the L isomer, which is easily assimilated during metabolism.

Lactic acid is mainly prepared in large quantities (around 200 kT per year) by the bacterial fermentation of carbohydrates. These fermentation processes can be classified according to the type of bacteria used: (i) the hetero-fermentative method, which produces less than 1.8 mol of lactic acid per mole of hexose, with other metabolites in significant quantities, such as acetic acid, ethanol, glycerol, mannitol and carbon dioxide; (ii) the homo-fermentative method, which leads to greater yields of lactic acid and lower levels of by-products, and is mainly used in industrial processes [3]. The conversion yield from glucose to lactic acid is more than 90 per cent.

The majority of the fermentation processes use species of *Lactobacilli* which give high yields of lactic acid. Some organisms predominantly produce the L isomer, such as *Lactobacilli amylophilus*, *L. bavaricus*, *L. casei* and

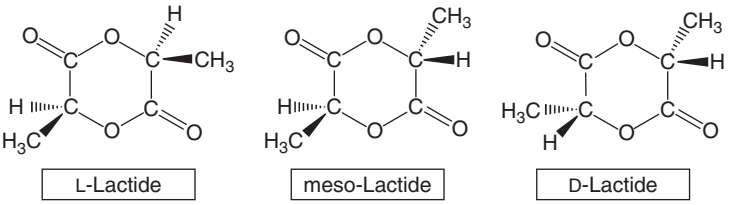

Figure 21.3 Chemical structures of L-, meso- and D-lactides.

L. maltaromicus, whereas, *L. delbrueckii*, *L. jensenii* or *L. acidophilus* produce the D isomer or a mixture of L and D [3.4]. These different bacteria are homo-fermentative. In general, the sources of basic sugars are glucose and maltose from corn or potato, sucrose from cane or beet sugar, etc. In addition to carbohydrates, other products, such as B vitamins, amino acids and different nucleotides, are formed. The processing conditions are an acid pH close to 6, a temperature around 40°C and a low oxygen concentration. The major method of separation consists in adding $CaCO_3$, $Ca(OH)_2$, $Mg(OH)_2$, NaOH, or NH_4OH to neutralize the fermentation acid and to give soluble lactate solutions, which are filtered to remove both the cells (biomass) and the insoluble products. The product is then evaporated, crystallized, and acidified with sulphuric acid to obtain the crude lactic acid. If the lactic acid is used in pharmaceutical and food applications, it is further purified to remove the residual by-products. If it is to be polymerized, it is purified by separation techniques including ultra-filtration, nano-filtration, electro-dialysis and ion-exchange processes.

21.2.1.2 Lactide

Figure 21.3 shows the different stereoforms of lactide. The cyclic dimer of lactic acid combines two of its molecules and gives rise to L-lactide or LL-lactide, D-lactide or DD-lactide, and meso-lactide or LD-lactide (a molecule of L-lactic acid associated with another one of D-lactic acid). A mixture of L- and D-lactides is a racemic lactide (rac-lactide). Lactide is usually obtained by the depolymerization of low molecular weight PLA under reduced pressure to give a mixture of L-, D- and meso-lactides. The different percentages of the lactide isomers formed depend on the lactic acid isomer feedstock, temperature and the catalyst's nature and content [3,4]. A key point in most of the processes is the separation between each stereoisomer to control the final PLA structure (*e.g.* by vacuum distillation) which is based on the boiling point differences between the meso- and the L- or D-lactide.

21.2.2 PLA polymerization

21.2.2.1 Lactic acid condensation and coupling

The condensation polymerization is the least expensive route, but it is difficult to obtain high molecular weights by this method. The use of coupling or esterification-promoting agents is required to increase the chains length [3, 4], but at the expense of an increase in both cost and complexity (multistep process). The role of chain coupling agents is to react with either the hydroxyl (OH) or the carboxyl end-groups of the PLA [3, 4, 7] thus giving telechelic polymers [10]. The nature of the chain end-groups should be fully controlled [2, 3]. The use of chain-extending agents brings some advantages, because reactions involving small amounts of them are economical and can be carried out in the melt without the need of separating the different process steps. The tunability to design copolymers with various functional groups is also greatly expanded. The disadvantages are that the final polymer may contain unreacted chain-extending agents, oligomers and residual metallic impurities from the catalyst. Moreover, some extending agents could be associated with a lack of biodegradability [2]. Examples of chain-extending agents are anhydrides, epoxides and isocyanates [11]. Similar products are used to develop compatibilization for PLA-based blends. The disadvantages of using isocyanates as chain extenders are their (eco)toxicity [3].

The advantages of esterification-promoting adjuvents are that the final product is highly purified and free from residual catalysts and/or oligomers. The disadvantages are higher costs due to the number of steps involved and the additional purification of the residual by-products [3], since these additives produce by-products that must be neutralized or removed.

21.2.2.2 Azeotropic dehydration and condensation

The azeotropic condensation polymerization is a method used to obtain high chain lengths without the use of chain extenders or adjuvents and their associated drawbacks. Mitsui Chemicals (Japan) has commercialized a process wherein lactic acid and a catalyst are azeotropically dehydrated in a refluxing, high boiling, aprotic solvent under reduced pressures to obtain high molecular weight PLA (Mw $\geq$ 300000) [2, 3]. A general procedure consists in the reduced pressure distillation of lactic acid for 2–3 h at 130°C to remove most of the condensation water. The catalyst and diphenyl ether are then added and a tube packed with molecular sieves is attached to the reaction vessel. The refluxing solvent is returned to the vessel by way of the molecular sieves during 30–40 h at 130°C. Finally, the ensuing PLA is purified [12].

This polymerization gives considerable catalyst residues because of its high concentration needed to reach an adequate reaction rate. This can cause many drawbacks during processing, such as degradation and hydrolysis. For most biomedical applications, the catalyst toxicity is a highly sensitive issue. The catalyst can be deactivated by the adding of phosphoric acid or can be precipitated and filtered out by the addition of strong acids such as sulphuric acid. Thus, residual catalyst contents can be reduced to some ppm [3].

21.2.2.3 ROP of lactide

The lactide method is the only method for producing pure high molecular weight PLA (Mw $\geq$ 100 000) [4, 6, 7, 13]. The ROP of lactide was first demonstrated by Carothers in 1932 [14], but high molecular weights were not obtained until improved lactide purification techniques were developed by DuPont in 1954 [2]. This polymerization has been successfully carried out calling upon various methods, such as solution, bulk, melt or suspension process. The mechanism involved in ROP can be ionic (anionic or cationic) or coordination–insertion, depending on the catalytic system [4, 6, 7, 13]. The role of the racemization and the extent of transesterification in the homo or copolymerization, are also decisive for the enantiomeric purity and chain architecture of the resulting macromolecules.

It has been found that trifluoromethane sulphonic acid and its methyl ester are the only cationic initiators known to polymerize lactide [15], and the mechanism of this process has been outlined in different papers [2, 3, 15].

Lactide anionic polymerizations proceed by the nucleophilic reaction of the anion with the carbonyl group and the subsequent acyl–oxygen bond cleavage, which produces an alkoxide end-group, which continues to propagate. The general mechanism for this anionic polymerization has been discussed in various publications [2, 3, 15, 16]. Some authors [16] have shown that the use of alkoxides, such as potassium methoxide, can yield well-defined polymers with negligible racemization.

Both the anionic and cationic ROPs are usually carried out in highly purified solvents, and although they show a high reactivity, they are susceptible to give racemization, transesterification and high impurity levels. For industrial and large commercial use, it is preferable to do bulk and melt polymerization with low levels of non-toxic catalysts. The use of less-reactive metal carboxylates, oxides and alkoxides has been extensively studied in this context, and it has been found that high molecular weight PLA can readily be obtained in the presence of transition metal compounds of tin [6, 7, 13], zinc [17, 18], iron [19] and aluminium [20], among others. A systematic investigation has led to the wide use of tin compounds, namely tin(II) bis-2-ethylhexanoic acid (stannous octoate) as a catalyst in PLA synthesis. This is mainly due to its high catalytic efficiency, low toxicity, food and drug contact approval and ability to give high molecular weights with low racemization [15]. The mechanisms of the polymerization with stannous octoate have been studied in detail, and it is now widely accepted that this ROP is actually initiated from compounds containing hydroxyl groups, such as water and alcohols, which are either present in the lactide feed or can be added upon demand. Figure 21.4 shows that the global mechanism is of the 'coordination–insertion' type [21], occurring in two steps: First, a complex between monomer and initiator is formed followed by a rearrangement of the covalent bonds; then, the monomer is inserted within the oxygen–metal bond of the initiator, and its cyclic structure is thus opened through the cleavage of the acyl–oxygen link, thus the metal is incorporated with an alkoxide bond into the propagating chain. It was found that the polymerization yield and the transesterification effect are affected by different parameters, such as the polymerization temperature and time, the monomer/catalyst ratio and the type of catalyst. The interaction between the time and temperature is very significant in terms of limiting the degradation reactions, which affect the molecular weight and the reaction kinetics [22]. It has also been shown that the chain length is directly controlled by the amount of OH impurities [23].

To make an economically viable PLA, Jacobsen *et al.* [21] developed a continuous one-stage process based on reactive extrusion with a twin-screw extruder. This technique requires that the bulk polymerization be close to completion within a very short time (5–7 min), which is predetermined by the residence time in the extruder.

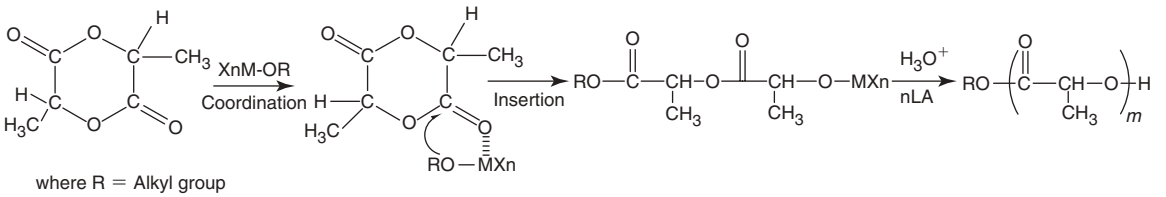

Figure 21.4 Coordination–insertion polymerization mechanism.

These authors showed that the addition of an equimolar content of a Lewis base, particularly triphenyl-phosphine, to stannous octoate increased the lactide polymerization rate.

21.2.3 Copolymers based on lactic acid units

A large number of macromolecular architectures of copolymers based on lactic acid have been investigated [7, 13]. Most of them are biodegradable or/and biocompatible. These copolymers can be prepared by using units containing a specific functionalized structure, thus giving rise to complex structure with unique properties. Examples of these materials are branched polyesters and graft copolymers (star, hyper-branched polymers) which involve different macromolecular architectures associated with novel materials properties and applications.

21.2.3.1 Ring-opening copolymerization

Several heterocyclic monomers can be used as comonomers with lactic acid in ring-opening copolymerizations, the most commonly used being glycolide (GA) for biomedical applications [24], caprolactone (CL) and valerolactone. The comonomer units can be inserted randomly or in block sequences.

21.2.3.2 Modification by high energy radiations and peroxides

Radical reactions applied to PLA to modify its structure have been generated by peroxides or high energy radiation [7]. Branching has been suggested to be the dominant structural change in poly(L-lactide) (PLLA) with peroxide concentrations in the range of 0.1–0.25 wt% and crosslinking above 0.25 wt% [7]. The peroxide melt-reaction with PLA has been found to cause strong modifications of the original PLA properties. A similar approach was recently developed with starch-based blends without any major improvement in their mechanical properties [25]. Irradiation of PLA causes mainly chain-scissions or crosslinking reactions, depending on the radiation intensity [26].

21.2.3.3 Graft copolymerization

Graft copolymers are often used as compatibilizers to improve the interfacial properties of blends or multiphase systems. Grafting reactions on a trunk polymer can be induced chemically, by plasma discharge, or by radiation (UV, X-rays or accelerated electrons), the latter approach giving purer products at high conversions. Plasma-induced grafting is performed by introducing an organic vapour into a plasma of inorganic gases to modify the surface properties of a substrate. Depending on the penetration depth of the irradiation, grafting can be performed either at the surface, or both on the skin and in the bulk [7].

The chemical modification of lactic acid-based polymers by graft copolymerization has been reported for the homopolymer of L-lactide and for copolymers with different L-lactide/CL contents [7, 13]. Carbohydrate polymers (*e.g.* amylose) can be modified by grafting lactic acid chains on their OH groups. A recent study [25] showed the interest of such a copolymer as a compatibilizer to improve the properties of starch/PLA blends to a better extent than the addition of peroxides or coupling agents (*e.g.* di-isocyanate) into the melt blend during the processing. Figure 21.5 shows the different steps involved in this grafting operation. After amylose purification to eliminate residual butanol and water, amylose-graft-PLA is obtained by the ROP of purified lactide with tin(II) bis (2-ethylhexanoate) in toluene at 100°C for 20 h.

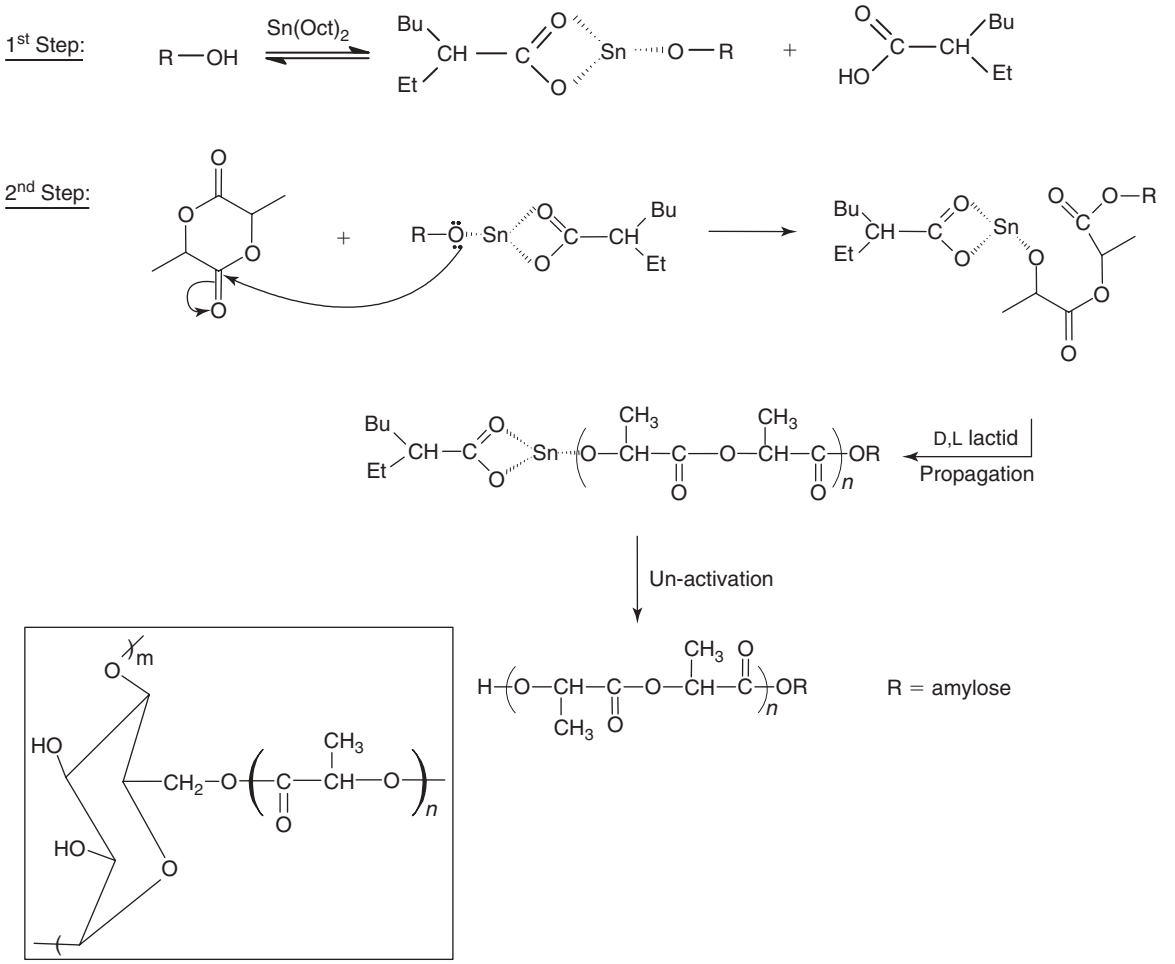

Figure 21.5 Mechanism of the ROP synthesis of amylose-graft PLA.

21.3 PLA PROPERTIES

21.3.1 Crystallinity and thermal properties

The properties of PLA, as indeed those of other polymers, depend on its molecular characteristics, as well as on the presence of ordered structures, such as crystalline thickness, crystallinity, spherulite size, morphology and degree of chain orientation. The physical properties of polylactide are related to the enantiomeric purity of the lactic acid stereo-copolymers. Homo-PLA is a linear macromolecule with a molecular architecture that is determined by its stereochemical composition. PLA can be produced in a totally amorphous or with up to 40 per cent crystalline. PLA resins containing more than 93 per cent of L-lactic acid are semi-crystalline, but, when it contains 50–93 per cent of it, it is entirely amorphous. Both meso- and D-lactides induce twists in the very regular PLLA architecture. Macromolecular imperfections are responsible for the decrease in both the rate and the extent of PLLA crystallization. In practise, most PLAs are made up of L-and D,L-lactide copolymers, since the reaction media often contain some meso-lactide impurities.

Table 21.1 gives the details of the different crystalline structures for neat PLA. Depending on the preparation conditions, PLLA crystallizes in different forms. The α-form exhibits a well-defined diffraction pattern [27]. This structure, with a melting temperature of 185°C, is more stable than its β-counterpart, which melts at 175°C [27].

Table 21.1

PLA crystalline structures. Unit cell parameters for non-blended PLLA and stereocomplex crystals.
(Adapted from Reference [8])

		Space group	Chain orientation	Number helices/ unit cell	Helical conformation	a (nm)	b (nm)	c (nm)	α (degrees)	β (degrees)	γ (degrees)
PLLA form	α	Pseudo-orthorhombic	–	2	103	1.07	0.645	2.78	90	90	90
PLLA form	α	Pseudo-orthorhombic	–	2	103	1.07	0.62	2.88	90	90	90
PLLA form	α	Orthorhombic	Parallel	2	103	1.05	0.61	–	90	90	90
PLLA form	β	Ort horhombic	–	6	31	1.031	1.821	0.90	90	90	90
PLLA form	β	Trigonal	Random up-down	3	31	1.052	1.052	0.88	90	90	120
PLLA form	γ	Orthorombic	Antiparallel	2	31	0.995	0.625	0.88	90	90	90
Stereo complex		Triclinic	Parallel	2	31	0.916	0.916	0.870	109.2	109.2	109.8

The latter form can be prepared at a high draw ratio and a high drawing temperature [28]. The γ-form is formed by epitaxial crystallization [29]. It has been observed that a blend with equivalent poly(L-lactide) PLLA and poly(D-lactide) PDLA contents gives stereo-complexation (racemic crystallite) of both polymers. This stereocomplex has higher mechanical properties than those of both PLAs, and a higher melting temperature of 230°C. The literature reports different density data [4] for PLA, with most values for the crystalline polymer around 1.29 compared with 1.25 for the amorphous material.

The crystallization kinetics of PLA have been extensively studied and found to be rather slow, as in the case of poly(ethylene terephthalate) PET. The rate of crystallization increases with a decrease in the molecular weight and is strongly dependent on the (co)polymer composition [4]. PLLA can crystallize in the presence of D-lactide [30], however, as the structure becomes more disordered, the rate of crystallization decreases. It has been reported that the crystallization rate is essentially determined by the decrease in the melting point of the different copolymers. PDLA/PLLA stereocomplexes are very efficient nucleating agents for PLLA, with increases in both the crystallization rate and the crystallinity, the latter of up to 60 per cent [31]. Quenching decreases the crystallization time [30]. As PET, PLA can be oriented by processing and chain orientation increases the mechanical strength of the polymer. If orientation is performed at low temperature, the resulting PLLA has a higher modulus without any significant increase in crystallinity. To determine the crystallinity levels by differential scanning calorimetry (DSC), the value most often referred to in the literature concerning the PLA melt enthalpy at 100 per cent crystallinity, is $93\,J\,g^{-1}$ [7, 8, 32] The crystallization of the thermally crystallizable, but amorphous PLA, can be initiated by annealing it at temperatures between 75°C and the melting point. Annealing crystallizable PLA copolymers often produces two melting peaks [32] and different hypotheses have been put forward to explain this feature. Yasuniwa *et al.* [33] found a double melting point in PLLA polymers and attributed them to slow rates of crystallization and recrystallization.

The typical PLA glass transition temperature (T_g) ranges from 50°C to 80°C, whereas its melting temperature ranges from 130°C to 180°C. For instance, enantiomerically pure PLA is a semi-crystalline polymer with a T_g of 55°C and a T_m of 180°C. For semi-crystalline PLA, the T_m is a function of the different processing parameters and the initial PLA structure. According to Ikada and Tsuji [30], T_m increases with increasing molecular weight (Mw) to an asymptotic value, but the actual crystallinity decreases with increasing Mw. T_m, moreover, decreases with the presence of meso-lactide units in its structure [4]. Both, the degree of crystallinity and the melting temperature of PLA-based materials can be reduced by random copolymerization with different comonomers (*e.g.* GA, CL or valerolactone).

The T_g of PLA is also determined by the proportion of the different types of lactide in its macromolecular chain.

21.3.2 Surface energy

Surface energy is critically important to many processes (printing, multilayering, etc.) and it influences the interfacial tension. The surface energy of a PLA made up of 92 per cent L-lactide and 8 per cent meson-lactide was found to be $49\,mJ\,m^{-2}$, with dispersive and polar components of 37 and $11\,mJ\,m^{-2}$, respectively [34], which suggests a relatively hydrophobic structure compared with that of other biopolyesters.

21.3.3 Solubility

A good solvent for PLA and for most of the corresponding copolymers is chloroform. Other solvents are chlorinated or fluorinated organic compounds, dioxane, dioxolane and furan. Poly(rac-lactide) and poly(meso-lactide) are soluble in many other organic solvents like acetone, pyridine, ethyl lactate, tetrahydrofuran, xylene, ethyl acetate, dimethylformamide, methyl ethyl ketone. Among non-solvents, the most relative compounds are water, alcohols (*e.g.* methanol, and ethanol) and alkanes (*e.g.* hexane and heptane) [7].

21.3.4 Barrier properties

Because PLA finds a lot of applications in food packaging, its barrier properties (mainly to carbon dioxide, oxygen and water vapour) have been largely investigated [4]. The CO_2 permeability coefficients for PLA polymers are lower than those reported for crystalline polystyrene at 25°C and 0 per cent relative humidity (RH) and higher than

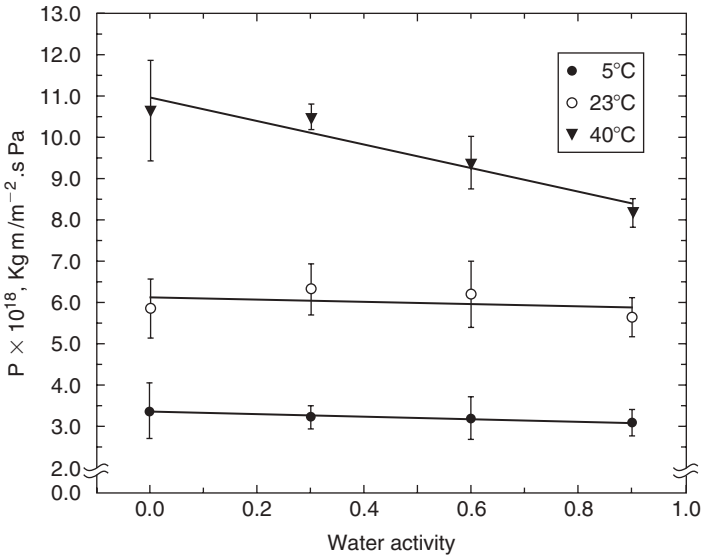

Figure 21.6 Oxygen permeability versus water activity at different temperatures, for poly(98 per cent L-lactide) films. (Source: Reference [4].)

those for PET. Since diffusion takes place through the amorphous regions of a polymer, an increase in the extent of crystallization will inevitably result in a decrease in permeability. Figure 21.6 shows the oxygen permeability for poly(98 per cent L-lactide) films as a function of the water activity. A significant increase in the oxygen permeability coefficient is shown as the temperature is increased, but, its decrease with water activity at temperatures close to T_g and its stabilization at temperatures well below T_g, are clearly visible. PET and PLA are both hydrophobic and the corresponding films absorb very low amounts of water, showing similar barrier properties, as indicated by the values of their water vapour permeability coefficient determined from 10°C to 37.8°C in the range of 40–90 per cent RH. Auras *et al.* [4] have shown that the permeability for 98 per cent L-lactide polymers is almost constant over the range studied, despite PLA being a rather polar polymer [4].

21.3.5 Mechanical properties

21.3.5.1 Solid state

The mechanical properties of PLA can vary to a large extent, ranging from soft and elastic materials to stiff and high strength materials, according to different parameters, such as crystallinity, polymer structure and molecular weight, material formulation (plasticizers, blend, composites, etc.) and processing (*e.g.* orientation). For instance, commercial PLA, such as poly(92 per cent L-lactide, 8 per cent meso-lactide), has a modulus of 2.1 GPa and an elongation at break of 9 per cent. After plasticization, its Young's modulus decreases to 0.7 MPa and the elongation at break rises to 200 per cent, with a corresponding T_g shift from 58°C to 18°C [32]. This example indicates that mechanical properties can be readily tuned to satisfy different applications.

The mechanical properties of PLA-related polymers were recently reviewed by Sodergard and Stolt [7], who showed, among other features, that the PLLA fibre modulus can be increased from 7–9 GPa to 10–16 GPa by going from melt to solution spinning. The mechanical behaviour can also be modified by preparing suitable copolymers, as in the case of the use of CL, which, with its soft segments, induces a decrease in modulus and an increase in the elongation at break, respectively.

21.3.5.2 Molten behaviour

For processing and for the corresponding applications, the knowledge of PLA melt rheology is of particular interest. A power law equation has been applied successfully by, for example, Schwach and Averous [34]. The pseudoplastic

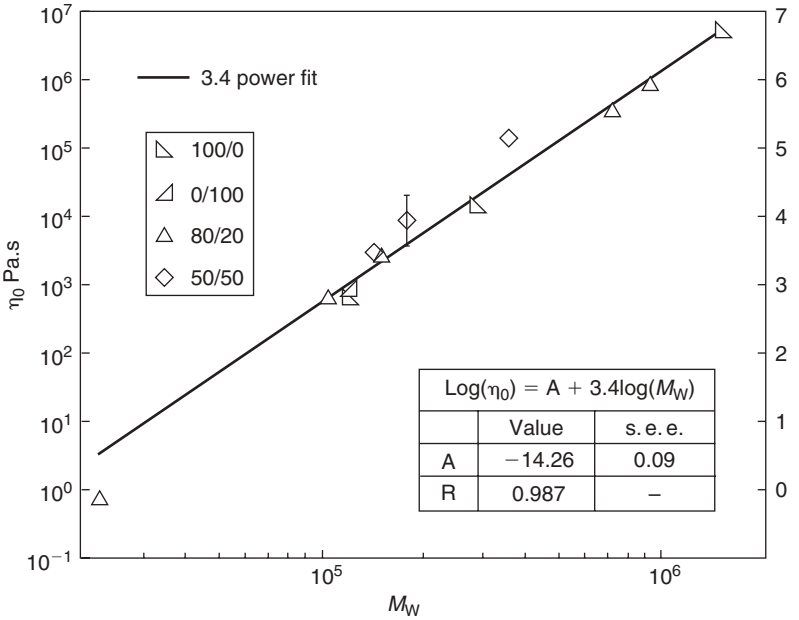

Figure 21.7 Zero-shear viscosity versus molecular weight for different L/D ratios (%). (Adapted from Reference [35].)

index is in the range 0.2–0.3, depending on the PLA structure. For instance, poly(92 per cent L-lactide, 8 per cent meso-lactide) displays a pseudoplastic index of 0.23. Figure 21.7, based on data published by Dorgan *et al.* [35], shows the evolution of the zero-shear viscosity versus molecular weight (Mw) for a wide range of L/D ratios (%), the latter parameter having virtually no effect. Static and dynamic characterizations have shown that the molecular weight between entanglements is around 10^4. Some other studies suggested that chain branching and molecular weight distribution have a significant effect on the melt viscosity of PLA [5].

21.4 DEGRADATION

21.4.1 Abiotic degradation

The main abiotic phenomena involve thermal and hydrolysis degradations during the life cycle of the material.

21.4.1.1 Thermal degradation

The thermal stability of biopolyesters is not significantly high, a fact that inevitably limits their range of applications. The PLA decomposition temperature is lies between 230°C and 260°C. Gupta and Deshmukh [36] concluded that the carbonyl carbon–oxygen linkage is the most likely bond to split under isothermal heating, as suggested by the fact that a significantly larger amount of carboxylic acid end-groups were found compared with hydroxyl end-groups. The reactions involved in the thermal degradation of lactic acid-based polymers can follow different mechanisms [7], such as thermohydrolysis, zipper-like depolymerization [36] in the presence of catalyst residues, thermo-oxidative degradation [37, 38] and transesterification reactions which give simultaneous bond breaking and bond making.

21.4.1.2 Hydrolytic degradation

PLA hydrolysis is an important phenomenon since it leads to chain fragmentation [4, 7, 39], and can be associated with thermal or biotic degradation. This process can be affected by various parameters such as the PLA structure, its molecular weight and distribution, its morphology (crystallinity), the shape of its samples and its thermal and mechanical history (including processing), as well as, of course, the hydrolysis conditions. Hydrolytic

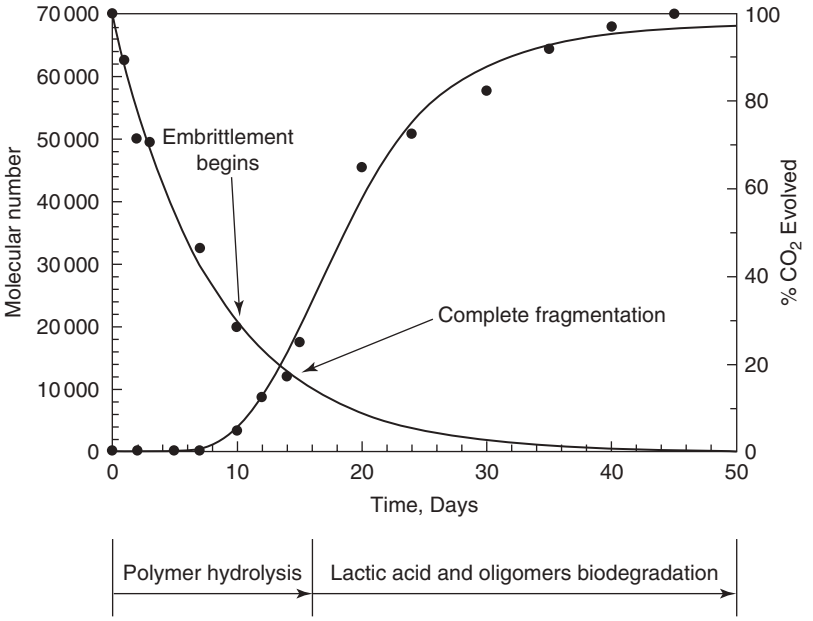

Figure 21.8 Abiotic and biotic degradations during composting stage. (Adapted from Reference [3].)

degradation is a phenomenon, which can be both desirable (*e.g.* during the composting stage) or undesirable (*e.g.* during processing or storage). The hydrolysis of aliphatic polyesters starts with a water uptake phase, followed by hydrolytic splitting of the ester bonds in a random way. The amorphous parts of the polyesters have been known to undergo hydrolysis before their crystalline regions because of a higher rate of water uptake. The initial stage is therefore located at the amorphous regions, giving the remaining non-degraded chains more space and mobility, which leads to their reorganization and hence an increased crystallinity. In the second stage, the hydrolytic degradation of the crystalline regions of the polyester leads to an increased rate of mass loss and finally to complete resorbtion [40]. The PLA degradation in an aqueous medium has been reported by Li *et al.* [40] to proceed more rapidly in the core of the sample. The explanation for this specific behaviour is an autocatalytic effect due to the increasing amount of compounds containing carboxylic end-groups. These low molar mass compounds are not able to permeate the outer shell. The degradation products in the surface layer are instead continuously dissolved in the surrounding buffer solution [40]. As expected, temperature plays a significant role in accelerating this type of degradation.

21.4.2 Biotic degradation

The biodegradation of aliphatic biopolyesters has been widely reported in the literature [5, 7, 39]. The biodegradation of lactic acid-based polymers for medical applications has been investigated in a number of studies *in vivo* [41] and some reports can also be found on their degradation in other biological systems [42]. The *in vivo* and *in vitro* degradations have been evaluated for PLA-based surgical implants [41]. *In vitro* studies have shown that the pH of the solution plays a key role in the degradation and that this analysis can be a useful predicting tool for *in vivo* PLA degradation [4]. Enzymes, such as proteinase K and pronase, have been used to bring about the *in vivo* PLA hydrolysis, although, enzymes are unable to diffuse through the crystalline parts. As expected, little enzymatic degradation occurs at the beginning of the process, but pores and fragmentation are produced, widening the accessible area to the different enzymes.

Figure 21.8 shows that during the composting stage, PLA degrades in a multistep process with different mechanisms [39]. Primarily, after exposure to moisture by abiotic mechanisms, PLA degrades by hydrolysis. First, random non-enzymatic chain-scissions of the ester groups lead to a reduction in molecular weight, with the consequent

embrittlement of the polymer. This step can be accelerated by acids or bases and is affected by both temperature and moisture levels [3]. Then, the ensuing PLA oligomers can diffuse out of the bulk polymer and be attacked by micro-organisms. The biotic degradation of these residues produces carbon dioxide, water and humus (mineralization).

Studies on PLA-based multiphase materials have been carried out. Gattin *et al.* [43] have found that the physical and morphological properties of the blend play an important role in its degradation behaviour, as in the case of their comparative study of the degradation of PLA with and without plasticized starch materials [43]. These authors reported that the nature of the degradation strongly depends on the experimental biodegradation conditions. Sinha Ray *et al*. [44] prepared PLA nano-biocomposites filled with montmorillonite and studied and characterized their biodegradability.

21.5 PROCESSING

21.5.1 Multiphase materials

The extrusion of PLA-based materials is generally linked with another processing step such as thermoforming, injection moulding, fibre drawing, film blowing, bottle blowing and extrusion coating. The properties of the polymer will therefore depend on the specific conditions during the processing steps (*e.g.* the thermomechanical input). The main parameters during the melt processing are temperature, residence time, moisture content and atmosphere [1]. But, the major problem in the manufacturing of PLA-based products is the limited thermal stability during the melt processing. To overcome such a drawback or to give PLA new properties, a large number of multiphase materials have been developed, mainly by mixing PLA with others products.

21.5.1.1 Plasticization

The brittleness and stiffness of PLA can be major drawbacks for some application. According to Ljungberg *et al.* [45], any factor influencing PLA crystallinity, such as the isomer ratio, could disturb the distribution and compatibility of plasticizers with PLA and induce low efficiency and phase separation.

Lactide monomer is an effective plasticizer for PLA, but presents high migration due to its small molecular size. Oligomeric lactic acid (OLA) seems to be a better answer, since it shows low migration and high efficiency [32]. For instance, adding 20 wt% of OLA into poly(92 per cent L-lactide, 8 per cent meso-lactide) induces T_g and modulus decreases of 20°C and 63 per cent, respectively. A significant improvement of PLA (mainly PLLA) flexibility is accomplished by the incorporation of different types of citrates [45, 48] or maleates [49] whose efficiency was evaluated in terms of T_g shift and mechanical properties improvement [32]. These plasticizers are miscible with PLA up to ~25 wt%, but increasing the plasticizer content can raise the PLA crystallinity by enhancing chain mobility [32]. Low molecular weight polyethylene glycol (PEG) [32], polypropylene glycol and fatty acid are also compatible with PLA and can act as plasticizers [5].

21.5.1.2 Blends and compatibilization

A great number of articles has been published during the last few decades on PLA-based blends [4, 5, 8, 32], including starch/PLA blends, which allow reducing the material cost without sacrificing its biodegradability and maintaining certain mechanical and thermal properties. Native starch, which is composed of semi-crystalline granules (see Chapter 15), can be physically blended with PLA, but remains in a separate conglomerate form in the PLA matrix [50]. Thus, starch is typically characterized as a solid filler with poor adhesion with PLA. Such biocomposites are used as a model to test (*e.g*. carbohydrate–PLA compatibilization [51]). Most of the studies which are focused on the production of starchy blends are based on plasticized starch, the so-called thermoplastic starch. Such a processable material is obtained by the disruption of the granular starch and the transformation of its semi-crystalline granules into a homogeneous, rather amorphous material with the destruction of hydrogen bonds between the macromolecules (for more details see Chapter 15). Disruption can be accomplished by casting (*e.g*. with dry drums) or by applying thermomechanical energy in a continuous process. The combination of thermal and mechanical inputs can be obtained by extrusion. After the processing, a homogeneous material is obtained [1, 32]. A dependence of the PLA glass transition temperature on the blend composition was observed by DSC and DMA, indicating a small degree of compatibility between the blend components [32]. However, the mechanical characteristics of the blends were modest. The blend morphology (discontinuous versus co-continuous) has been

investigated by Schwach and Averous [34] by microscopic observations. The full co-continuity is obtained in the domain of 60–80 per cent in volume of PLA. Despite the interest in developing plasticized starch/PLA materials, some limitations, due to the lack of affinity between the respective constituents, seem difficult to overcome. This low compatibility is mainly due to the PLA hydrophobic character.

To improve the affinity between the phases, compatibilization strategies are generally developed. This implies the addition of a compound, the compatibilizer, which can be obtained by the modification of at least one of the polymers initially present in the blend. For PLA/starch compatibilization, the literature proposes different approaches which can be classified in four groups [1, 25]: (i) the functionalization of PLA with, for example, maleic anhydride [51]; (ii) the functionalization of starch with, for example, urethane functions [25]; (iii) the starch–polyester crosslinking with a coupling agent such as a peroxide [25] and (iv) the use of copolymers, for example, starch-graft PLA [25], following the mechanism discussed above and illustrated in Fig. 21.5, for which the length of the grafts can be controlled to obtain a comb structure [52].

It is known that PLA forms miscible blends with polymers such as PEG [53]. PLA and PEG are miscible with each other when the PLA fraction is below 50 per cent [53]. The PLA/PEG blend consists of two semi-miscible crystalline phases dispersed in an amorphous PLA matrix. PHB/PLA blends are miscible over the whole range of composition. The elastic modulus, stress at yield, and stress at break decrease, whereas the elongation at break increases, with increasing polyhydroxybutyrate (PHB) content [54]. Both PLA/PGA and PLA/PCL blends give immiscible components [55], the latter being susceptible to compatibilization with P(LA-*co*-CL) copolymers or other coupling agents.

21.5.1.3 Multilayers

Developing compostable and low cost multilayer materials based for instance on plasticized starch and PLA is interesting in more than one sense. Martin *et al.* [56] carried out several studies on such a system and showed that the basic requisites for the preparation of multilayered products are to obtain sufficient adhesion between the layers, good moisture barrier properties and a uniform layer thickness distribution. Two different techniques were used to prepare the multilayers, namely, coextrusion and compression moulding. Peel strength was controlled by the compatibility between plasticized starch and PLA, which stayed low without compatibilizer. It was possible to increase the adhesion properties of the film by up to 50 per cent (*e.g.* by blending low polyester contents into the starchy core layer). There exist some inherent problems due to the multilayer flow conditions encountered in coextrusion, such as encapsulation and interfacial instability phenomena [57]. Addressing these problems is a crucial issue, since they can be detrimental to the product, affecting its quality and functionality.

21.5.1.4 Biocomposites and nano-biocomposites

Different types of fillers have been tested with PLA, such as calcium phosphate or talc [58], which show an increase in its mechanical properties. Concerning inorganic fillers, the greatest reinforcing effect is obtained with whiskers of potassium titanate and aluminium borate with a high aspect ratio. Carbon or glass fibres [59] improve the mechanical properties, particularly with fibre surface treatments capable of inducing strong interactions with PLA matrix. Different organic fillers can be associated with PLA. Biocomposites with improved mechanical properties are obtained by the association of ligno-cellulose fillers, such as paper-waste fibres and wood flour, with PLA by extrusion and compression moulding.

A significant and increasing number of papers have been published during the last 5 years on nano-biocomposites (*i.e.* nanocomposites based on a biodegradable matrix). Polylactide/layered silicate nanocomposites were largely investigated by Sinha Ray *et al.* [60, 61] and other authors [62–63]. They successfully prepared a series of biodegradable PLA nano-biocomposites using mainly melt extrusion of PLA, principally with modified montmorillonites (O-MMT), targeting nanofillers exfoliation into the matrix. Because of the interactions between the organo-clay particles which present large surface area (several hundreds m^2g^{-1}) and the PLA matrix, the nano-biocomposites dislayed improved properties, such as mechanical moduli, thermal stability, crystallization behaviour, gas barrier and biodegradability. The preparation of biodegradable nanocellular polymeric foams via nanocomposites technology based on PLA and layered silicate has been reported by different authors [61, 64] who used supercritical carbon dioxide as a foaming agent, with the silicate acting as nucleating site for cell formation. Cellular PLA structures can also be obtained by producing a co-continuous structure and extracting the co-products [65].

21.6 APPLICATIONS

At present, PLA-based materials are mainly referenced on three different markets, namely, the biomedical (initial market), the textile (mainly in Japan) and the packaging (mainly food, *i.e*. short-term applications). For instance, reported types of manufactured products are blow-moulded bottles, injection-moulded cups, spoons and forks, thermoformed cups and trays, paper coatings, fibres for textile industry or sutures, films and various moulded articles [8].

21.6.1 Biomedical applications

PLA has been widely studied for use in medical applications because of its bioresorbability and biocompatible properties in the human body. The main reported examples on medical or biomedical products are fracture fixation devices like screws, sutures, delivery systems and micro-titration plates [8].

PLA-based materials are developed for the production of screws and plates. As the bone healing progresses, it is desirable that the bone is subjected to a gradual increase in stress, thus reducing the stress-shielding effect. This is possible only if the plate loses rigidity in *in vivo* environment. To meet this need, researchers introduced resorbable polymers for bone plate applications. PLA resorbs or degrades upon implantation into the body, but most of its mechanical properties are lost within a few weeks [41]. Tormala *et al*. [66] proposed fully resorbable composites by reinforcing matrices with resorbable PLLA fibres and calcium phosphate-based glass fibres. One of the advantages often quoted for resorbable composite prostheses is that they do not need to be removed with a second operative procedure, as with metallic or non-resorbable composite implants. To improve the mechanical properties, PLA is reinforced with variety of non-resorbable materials, including carbon and polyamide fibres. Carbon fibres/PLA composites possess very high mechanical properties before their implantation, but they lose them too rapidly *in vivo* because of delamination. The long-term effects of resorbed products, and biostable or slowly eroding fibres in the living tissues are not fully known, and are concerns yet to be resolved [41].

Although PLA fibres are used in different textile applications as, for example, non-woven textile for clothes, they achieved their first commercial success as resorbable sutures. One of the first commercially available fibre-formed bioresorbable medical products is based on copolymers of GA in combination with L-lactide (Vicryl) [67]. Fibres can be produced both by solvent and by melt-spinning processes and drawn under different conditions to orient the macromolecules [7].

Micro- and nanoparticles are an important category of delivery systems used in medicine, and the use of PLA is interesting due to its hydrolytic degradability and low toxicity. The most important properties of the micro- and nanoparticles are the drug release rate and the matrix degradation rate which are affected by the particle design and the material properties [7]. Copolymers of GA and rac-lactide [5] seem to be the most suitable combinations for use as drug delivery matrices.

Porous PLA scaffolds have been found to be potential reconstruction matrices for damaged tissues and organs. There are several techniques reported for the manufacturing of such materials [7].

21.6.2 Packaging applications

Commercially available PLA packaging can provide better mechanical properties than polystyrene and have properties more or less comparable to those of PET [4, 8]. Market studies show that PLA is an economically feasible material for packaging. With its current consumption, it is at the present the most important market in volume for biodegradable packaging [4, 8]. Due to its high cost, the initial use of PLA as a packaging material has been in high value films, rigid thermoforms, food and beverage containers and coated papers. One of the first companies to use PLA as a packaging material was Danone (France) in yoghurt cups for the German market. During the last decade, the use of PLA as a packaging material has increased all across Europe, Japan and the US, mainly in the area of fresh products, where PLA is being used as a food packaging for short shelf-life products, such as fruit and vegetables. Package applications include containers, drinking cups, sundae and salad cups, wrappings for sweets, lamination films, blister packages and water bottles [9, 68]. Currently, PLA is used in compostable yard bags to promote national or regional composting programs. In addition, new applications such as cardboard or paper coatings are being pursued, for example, the fast-food market (cups, plates and the like) [9, 68]. However, to cater for a larger market, some PLA drawbacks must be overcome, such as its limited mechanical and barrier properties and heat resistance, and, in order to meet market expectations, the world production of PLA must be substantially increased.

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Polyhydroxyalkanoates: Origin, Properties and Applications

Ivan Chodak

ABSTRACT

This chapter deals with polyhydroxyalkanoates (PHAs), a class of biodegradable polyesters produced by various bacteria, but which can also be synthesized chemically. After a historical introduction, the bacterial synthesis and the structure of polyhydroxybutyrate and its copolymers with various homologous monomers are reviewed, before tackling various aspects related to the properties and behaviour of these PHAs. In particular, the mechanical and thermal properties, as well as the physical ageing, are placed in the context of possible applications. Finally, different processing technologies are examined together with the possibility of preparing plasticized or blended materials. Emphasis is also placed on the problem of economic competitivity of these polymers, compared with petroleum-based counterparts.

Keywords

Polyhydroxyalkanoates, Polyhydroxybutyrate, History, Bacterial synthesis, Chemical synthesis, Genetic engineering, Mechanical properties, Thermal transitions, Crystallization, Plasticizers, Thermal degradation, Processing, Applications

22.1 INTRODUCTION

Polyhydroxyalkanoates (PHAs) represent an important group of biodegradable plastics. They are produced by various bacteria in many grades, differing in composition, molecular weight and other parameters [1, 2]. The formation of a particular material, either homo or copolymer, depends on the type of bacteria, but even more important are the conditions of polymer formation, mainly the substrate used for feeding the bacteria and the conditions of their growth.

PHAs are generated and stored in bacteria in the form of fine powder particles. The bacteria produce the PHAs as an energy reserve, that is when overfed, the polymer is formed, which is consumed when the bacteria is short of a feeding substrate.

The simplest PHA is polyhydroxybutyrate (PHB), whose formula is shown in Fig. 22.1. This polymer and its copolymer with polyhydroxyvalerate seem to be, at present, the only PHAs relevant for practical applications.

PHB can be characterized as a rather controversial polymer. It is produced from renewable resources via a classical biotechnological process. The polymer is completely biodegradable, highly hydrophobic and thermoplastic, with high crystallinity, high melting temperature, good resistance to organic solvents and possesses excellent mechanical strength and modulus, resembling that of polypropylene [3]. In spite of these excellent properties, especially the strength parameters, the application of the polymer is limited to small volumes for rather special purposes. High volume applications are hindered by several serious drawbacks, especially pronounced brittleness, very low deformability, high susceptibility to a rapid thermal degradation, difficult processing by conventional

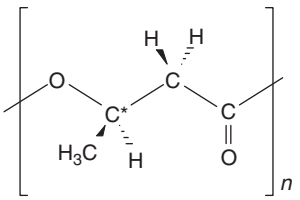


Figure 22.1 The formula of simplest PHA, polyhydroxybutyrate (PHB).

thermoplastic technologies (mainly due to fast thermal degradation) and rather high price compared to other high volume plastics. Additional problems related to processing are connected mainly with low shear strength of the melt, which needs to be addressed when considering certain applications. Its copolymer with valerate (PHBV) is much more acceptable, especially regarding improved toughness with an acceptable loss of strength and modulus. However, until now, the production process of PHBV has been more demanding, and hence its price is even higher than that of PHB. In spite of this, PHAs are considered to be promising materials worthy of being broadly investigated, and their production on a large scale is being considered or prepared in several regions of the world.

22.2 HISTORICAL REVIEW

The occurrence of PHA in bacterial cells was first described by Beijerinck in 1888 [4]. However, PHAs were studied mainly by biochemists who referred to them as 'lipids' [5], until the determination of the PHA composition by Lemoigne in 1925 [6, 7], who showed that the unknown material produced by *Bacillus megaterium* was a homopolyester of hydroxyacid, poly-3-hydroxybutyrate. That bacteria could produce polyesters was unknown to polymer chemists before 1960 and even to most biochemists and microbiologists before 1958, although their presence in bacterial cells in isolable amounts, their chemical composition, and even the fact that they were polymers, were reported in the literature as early as 1926. Generally, the scientific community did not realize the existence of these natural polyesters for so long because their discoverer, Maurice Lemoigne, published his results in little-read French journals and referred to the new material as ether-insoluble lipids [8]. At that time, many organic chemists refused to believe that there were such things as polymers. Lemoigne was a bacteriologist with training in analytical chemistry, and his series of papers, published over the 5-year period from 1923 to 1927, are remarkable for their breadth of research [9–13]. In 1923, Lemoigne reported that the acid produced by the bacteria was 3-hydroxybutyric acid [9], and in 1927, he described the procedure of obtaining the material from the cell which he characterized as a polymer of 3-hydroxybutyric acid. He named the source of the acid *lipide*-β-*hydroxybutyrique*, and also determined its molecular weight and melting temperature.

As pointed out by Lenz and Marchessault in an excellent review on PHAs [8], Lemoigne published these observations and interpretations at the time when Herman Staudinger was proposing the existence of high molecular weight molecules or polymers, which he termed 'macromolecules'. Lemoigne was probably familiar with the work of Emil Fischer, who had demonstrated as early as 1906 that proteins are large molecules of 'enchained' amino acid units or 'polypeptides', as he was the first to call them [14]. In 1953, Staudinger was awarded the Nobel Prize in Chemistry for his work on polymer synthesis and the concept of macromolecules [15]. There is no indication that Staudinger was aware of Lemoigne's discovery of nature's polyesters, which remained hidden from organic and polymer chemists for over 30 years, even though PHB was described in biochemistry textbooks, where, however, it was referred to as a 'lipid', and not polyester.

From 1923 until 1951, Lemoigne and co-workers published 27 papers. Lemoigne worked out a method for the quantitative analysis of PHB, and showed that it could be cast into a transparent film [16]. It was also reported that a variety of bacteria could produce PHB, and Lemoigne labelled it a 'reserve material'. It was only in the late 1950s that the important role of PHB in the overall metabolism of bacterial cells was discovered, understood and the significance of Lemoigne's earlier discoveries realized [8] by Stanier and Wilkinson and their co-workers, who showed that the PHB granules in bacteria serve as an intracellular food and energy reserve and that the polymer is produced by the cell in response to a nutrient limitation in the environment in order to prevent starvation if an essential element becomes unavailable. The nutrient limitation activates a metabolic mechanism, in which acetyl

units from the tricarboxylic cycle are involved in the production of PHB. This can be reconverted into acetic acid by a series of enzymatic reactions inside the cell [17].

In 1958, Macrae and Wilkinson observed that the production of the homopolymer by *B. megaterium* was rather fast if the glucose-to-nitrogen source ratio of the substrate was high [18] and the ensuing degradation by *B. Cereus* or *B. Megaterium* occurred rapidly in the absence of any energy source [19, 20]. The authors concluded that PHB was a carbon- and energy-reserve material and suggested the pathway of PHB synthesis [19]. In the same year, it was also demonstrated that the occurrence of this reserve polymer is a widespread phenomenon in Gram-negative bacteria [21]. During the same period, Stanier, Doudoroff and co-workers found that PHB was the primary product of the oxidative and photosynthetic assimilation of organic compounds by phototropic bacteria, and they attempted to detail the biosynthesis and breakdown mechanism of PHB in the cells [22]. The reactions involved in the metabolic pathway responsible for the biosynthesis of PHB from acetic acid were first identified by Stanier and co-workers in 1959 in their studies on PHB formation in *R. rubrum*. However, only in 1973, Schlegel at the University of Gottingen, and Dawes at the University of Hull, working independently, succeeded to isolate and characterize the specific enzymes which catalyze the reactions for the synthesis of 3-hydroxybutyric acid, the PHB monomer [23–26]. Schlegel *et al.* carried out their investigations on PHB production in *Alcaligenes eutrophus*, while Dawes and co-workers studied the cycle in *Azotobacter beijerinckii*. In the same 1973 issue of *Biochemical Journal*, Schlegel and Dawes published simultaneously their discoveries on the identification of the two enzymes involved in the reactions for converting acetic acid into 3-hydroxybutyric acid in the two different bacteria [23, 24]. For both bacteria, the enzymes were a ketothiolase, which catalyzes the dimerization of the Coenzyme A derivative of acetic acid, acetyl-CoA, to acetoacetyl-CoA, and a reductase, which catalyzes the hydrogenation of the latter to [*R*]-3-hydroxybutyryl-CoA, the monomer that is polymerized to PHB by a synthase [8].

A detailed mechanism for the polymerization reaction, which was based on Merrick's suggestion, was proposed by Ballard and co-workers at ICI in 1987 and further elaborated by Doi and co-workers at the RIKEN Institute in Japan in 1992. They put forward a mechanism in which two thiol groups are involved in the active site for both the initiation and propagation reactions of the polymerization. For initiation, the two thiol groups form thioesters with two molecules of monomer, which then undergo a thioester–oxyester interchange reaction at the active site to form a dimer and release one of the thiol groups for the propagation reaction [8].

3-Hydroxybutyrate (3HB) was, for a long time, considered to be the only PHA material produced by bacteria as the reserve energy supply until, in 1974, Wallen and Rohwedder identified 3-hydroxyvalerate and 3-hydroxyhexanoate in chloroform extracts of activated sewage sludge [27]. The possibility of preparing various PHAs as a function of the substrate was first revealed by DeSmith *et al.* [28] when they cultivated *Pseudomonas oleovorans* in *n*-octane. The polymer formed was found to consist principally of 3-hydroxyoctanoate units.

In the 1970s, the interest in PHAs was rising, as reflected in a growing number of papers dealing with various aspects of these polymers. On the one hand, many different microorganisms were found to be able to produce PHAs, as described in an early review on PHAs by Daves and Senior in 1973 [29]. On the other hand, the widening research of polymer scientists on PHAs resulted in a better characterization of the materials regarding their macromolecular parameters, such as molecular weight [30], crystalline structure [31], granule morphology and other properties [32–34]. Continuing work on biochemical aspects led to a detailed knowledge on the PHB metabolism and its regulation [29, 35], the enzymology of PHB synthesis [29], the PHB intra- and extra-cellular degradation [4, 36] and the physiological function of PHB. The recognition of the relationship between PHB biosynthesis and extra-cellular environment was of particular importance, especially since it made it possible to optimize the preparation process of PHB via defining the conditions that favour PHB accumulation [24, 37]. From a practical point of view, the identification of HA units, other than 3HB, and especially the possibility to produce copolymers, revealed a way to envisage practical applications for these polymers, since it solved the serious problem of PHB brittleness. Thus, the first industrial production of PHB-*co*-valerate took place [38], although the patents on 3PHB as a potentially biodegradable plastic had already been filed in 1962 [39]. In the 1980s, extensive work was done to identify all potential HA units resulting, not only in the description of a wide range of 3HA units, but also in the discovery of 4HA [40] and 5HA [41] units in the polymer chain.

To extend the vast flexibility of nature regarding PHA production, it was found that these materials are synthesized, not only by Gram-negative bacteria, but also in a number of Gram-positive counterparts, bacteria, aerobic, anaerobic, photosynthetic bacteria, as well as in some archaebacteria [42, 43].

In 1982, Imperial Chemical Industries Ltd. (ICI) in England started to produce a thermoplastic polyester that was totally biodegradable and could be melt processed including the preparation of films and fibres [42]. The technology consisted in a large-scale fermentation process, resulting in the production of the polymer inside

the bacterial cells, which at the end of the process contained as much as 90 per cent of the polymer. The bacterium employed in the process was *Alcaligenes eutrophus*, since then renamed *Ralstonia eutropha* (and more recently changed again to *Wautersia eutropha*) and the commercial polyester product with the trade name 'Biopol', was a copolyester containing randomly arranged units of [*R*]-3-hydroxybutyrate (HB), and [*R*]-3-hydroxyvalerate (HV) [44].

The fast development of molecular biology, starting in the late 1970s led to an investigation of the cloning and characterization of genes involved in the PHA biosynthesis. At the end of the 1980s, the genes coding for the enzymes involved in the PHA synthesis were cloned from *Ralstonia eutropha* and shown to be functionally active in *Escherichia coli* [45–47]. Thus, what was identified at the beginning of the twentieth century as a sudanophilic bacterial inclusion is now apparently going to the most advanced stage of development, that is, protein engineering [3]. This development may lead in the near future to an efficient production of tailor-made PHAs with a high potential for environment-friendly applications.

22.3 PREPARATION, SYNTHESIS

Bacterial synthesis is considered to be the most important process for PHA preparation at present. This approach will be discussed in this section in more detail. Besides the bacterial synthesis, the chemical synthesis of PHB via the ring-opening polymerization of butyrolactone, is also possible and will be briefly discussed at the end of this section. Recently, increased attention has been paid to the biosynthesis using genetically modified plants. This procedure has not yet reached an industrially acceptable scale up, but may be developed to an even more efficient and economical process than the bacterial fermentation technology, as discussed below.

22.3.1 Bacterial synthesis

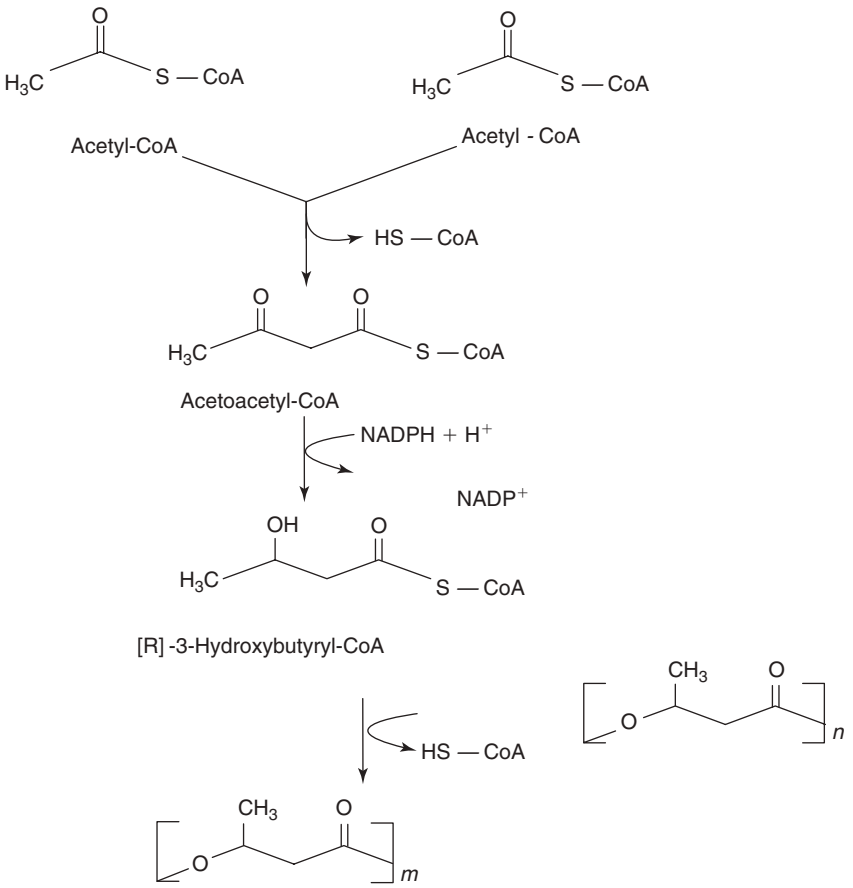
As described above, the bacterial synthesis was the first, natural way to prepare and produce PHAs. A lot of work was devoted to unravel principles, mechanisms and possibilities to modify the process [1, 3], so that now the process is basically understood and the principles are known, enabling its modification to produce the desired products.

The first step of the biochemical pathway of PHB synthesis consists in the conversion of a selected carbon source to acetate. Then, an enzyme cofactor is attached via the formation of a thioester bond. The enzyme, called coenzyme A (CoA), is a universal carrier of acyl groups in biosynthesis and acetyl-CoA is a basic metabolic molecule found in all PHA-producing organisms. A dimer acetoacetyl-CoA is formed via reversible condensation and subsequently reduced to a monomer unit (R)-3-hydroxybutyryl-CoA. PHB is formed via the polymerization of the latter, maintaining the asymmetric centre [5]. The basic simplified process is shown in Scheme 22.1.

Propagation proceeds by bonding another monomer to the free thiol group of the active site, followed by another thioester–oxyester exchange reaction to form the trimer, and so on. The higher bond energy of the oxyester, compared to that of the thioester, is the reason for polymerization proceeding spontaneously as a thermodynamically favourable process. The synthase, therefore, acts as both initiator and catalyst for the polymerization process, which proceeds by a continuous series of insertion reactions. The enzyme is specific for monomers with the [*R*] configuration and will not polymerize identical compounds having the [*S*] configuration. Hence, all natural PHAs are totally isotactic.

It has been demonstrated that PHB is accumulated by a wide variety of bacteria under unbalanced growth, when the cells become limited for an essential nutrient (such as ammonium, sulphate, phosphate, magnesium, iron) but are exposed to an excess of carbon. Under conditions of balanced growth, CoA amounts are relatively high, so that the synthesis of PHB is inhibited and acetyl-CoA is just metabolized in the tricarboxylic acid cycle. In nutrient limitation without carbon excess, proteins cannot be synthesized, and the buildup of NADH, an universal electron carrier in biosynthesis, occurs. NADH inhibits citrate synthesis and consequently acetyl-CoA is no longer able to be oxidized rapidly enough via the tricarboxylic acid cycle and therefore accumulates. Although the equilibrium constant of the reversible condensation reaction does not favour acetoacetyl-CoA formation, at high concentrations of NADH and acetyl-CoA at low concentrations of CoA, the equilibrium is shifted in favour of the PHB biosynthesis [5].

As listed in Table 22.1, a number of bacterial genera produce PHAs, mainly PHB, under various conditions with different yields. Various microorganisms produce PHAs with different levels of efficiency and quality. However, the



Scheme 22.1 Bacterial synthesis of PHB.

number of strains which are able to synthesize polymers intracellularly is surprising. A limited number of bacteria are able to produce random copolymers based on the usual 3HA and 3HV combination. Initially, *A. eutrophus* was grown on a variety of substrates, including natural sugar, ethanol and even gaseous mixtures of carbon dioxide and hydrogen [48] to synthesize PHB. Later, it was found that the HV comonomer could be incorporated if propionate or valerate were present in the medium [49]. The HV mole fractions were limited to the range 0–47 per cent, because of the relatively fast metabolic pathway of propionyl-CoA to acetonyl-CoA in the cell [50, 51]. Later on, a series of copolymers with 0–95 per cent of HV were produced from mixtures of butyric and valeric acids [52]. The direct incorporation of 4-hydroxybutyric acid [41, 53, 54] and 3-hydroxypropionic acid was also found to be possible, for example, a random copolyester of 3-HB and 4-HB units containing 37 per cent of 4-HB was produced when 4-hydroxybutyric acid was used as the sole carbon source [41].

The large number of bacteria able to produce PHAs is impressive. However, even more surprising is the variety of substrates which the bacteria are able to consume, producing, besides PHB and PHB-*co*-HV, a broad range of polymers, copolymers, terpolymers, including polymers containing various functional groups, such as double bonds, halogens, phenyl or epoxy moieties, depending on the type of the strain and substrate the bacteria are grown on. From this point of view, *Pseudomonas genus* has been reported as the most versatile accumulator of PHAs [37]. A brief overview of syntheses using bacteria of this type is shown in Table 22.2. The examples of some more or less special syntheses with other bacteria are listed in Table 22.3.

Among those, besides the PHB homopolymer, poly-3-hydroxybutyrate copolymers with either 3-hydroxyvalerate or 4-hydroxybutyrate are of special interest, mainly because these copolymers, while having a lower crystallinity

Table 22.1

Bacterium species accumulating PHA. Data taken from [5] and [37]

1. Acinetobacter	28. Gloeothece	55. Protomonas
2. Actinomycetes	29. Haemophilus	56. Pseudomonas
3. Alcaligenes[a]	30. Halobacterium	57. Ralstonia[a]
4. Aphanocapsa	31. Haloferax	58. Rhizobium
5. Aphanothece	32. Hyphomicrobium	59. Rhodobacter
6. Aquaspirillum	33. Lamprocystis	60. Rhodococcus
7. Asticcaulus	34. Leptothrix	61. Rhodopseudomonas
8. Azomonas	35. Lampropedia	62. Rhodospirillum
9. Azospirillum	36. Methanomonas	63. Sphaerotilus
10. Azotobacter	37. Methylobacterium	64. Spirillum
11. Bacillus	38. Methylocystis	65. Spirulina
12. Beggiatoa	39. Methylomicrobium	66. Staphylococcus
13. Beijerinckia	40. Methylomonas	67. Stella
14. Beneckea	41. Methylosinus	68. Streptomyces
15. Caryophanon	42. Methylovibrio	69. Syntrophomonas
16. Caulobacter	43. Micrococcus	70. Tetrahymena
17. Chlorofrexeus	44. Microcoleus	71. Thiobacillus
18. Chlorogloea	45. Microcystis	72. Thiocapsa
19. Chromatium	46. Moraxella	73. Thiocystis
20. Chromobacterium	47. Mycoplana	74. Thiodictyon
21. Clostridium	48. Nitrobacter	75. Thiopedia
22. Corynebacterium	49. Nitrococcus	76. Thiosphaera
23. Derxia	50. Nocardia	77. Vibrio
24. Ectothiorhodospira	51. Oceanospirillum	78. Wautersia[a]
25. Escherichia	52. Paracoccus	79. Xanthobacter
26. Ferrobacillus	53. Pedomicrobium	80. Zoogloea
27. Gamphospheria	54. Photobacterium	

[a] Different names used for the same bacteria. Wautersia has been recently used.

which could be important regarding toughness, might be produced by technologies acceptable from an economical point of view. *Ralstonia eutropha*, *Alcaligenes latus* or *Delftia acidovorans* were shown to produce random copolymers of 3-hydroxybutyric acid (3HB) and 4-hydroxybutyric acid (4HB), P(3HB-*co*-4HB), in a broad composition range using various carbon sources [41, 63, 67, 71]. When 4-hydroxybutyric acid, or 1,4-butanediol, was used as the sole carbon source for *D. acidovorans*, the homopolymer of 4-hydroxybutyric acid (P(4HB)) was synthesized [67, 82].

The isolation of these polymers from the biomass proceeds through the destruction of the cell membrane mechanically, chemically or eznymatically [42, 83], followed by the dissolution of the polymer in a suitable solvent, for example chloroform, methylene chloride, 1,2-dichloro ethane or pyridine. The remnants of the cell walls are removed by filtration and/or centrifugation. Extraction, using mixed solvents, for example water/organic solvent, is the last step applied for the final purification.

22.3.2 Chemical synthesis

It should be pointed out that all of the PHAs can be synthesized chemically from the relevant substituted propiolactones, generally using aluminium or zinc alkyl catalysts with water as the cocatalyst. Several alternative routes have been explored, for example PHBV can be produced from butyrolactone and valerolactone with an oligomeric aluminoxane catalyst [85]. Structures with partially stereoregular blocks can also be obtained. An interesting and instructive review on the chemical synthesis was published by Muller and Seebach in 1993 [84].

Table 22.2

Overview of PHAs produced by *Pseudomonas genus* on various substrates. Data taken from [37]

Bacteria	PHA produced	Substrates	Special feature	Reference
Pseudomonas oleovorans	4 to 12 C monomers (R═CH_3 to $(CH_2)_8CH_3$)	*n*-Alkanes, *n*-alkanoates, *n*-alcohols, *n*-alkenes – unsaturated monomers	Branched side chains from branched substrates	[42]
	Units contain 2 C less than the C source		Composition related to substrate	[55]
	Unsaturated medium-side-chain	*n*-Octane + *n*-octene		[55]
	PHAs containing phenyl units	Mixture of 5-phenylvaleric acid + *n*-nonanionic acid or *n*-octanionic acid	Low yield	[56]
	Copolymer containing 32% of cyano containing monomers (9-cyano-3-hydroxynonanoate and 7-cyano-3-hydroxyheptanoate	11-cyanoundecanoic acid + *n*-nonanionic acid		[57]
	Chlorinated and fluorinated PHAs			[58, 59]
	Brominated units	Nonanionic and octanionic acids + 6-bromohexanoic, 8-bromo-octanoic acid or 11-bromo-undecanoic acid		[59]
	Copolyester containing up to 37% of terminal epoxy groups	Mixture of 10-epoxyundecanoic acid and sodium octanoate		[60]
Pseudomonas putida	Seven different comonomers including 3-hydroxy decanoate, 3-hydroxybexanoate, octanoate + saturated and monounsaturated monomers of C12 and C14	Glucose		[61]
Pseudomonas sp. A33	Unsaturated medium-side-chains			[62]
	3HB + nine other components including 3-hydroxyhexadecanoate + unsaturated 3-hydroxydodecenoate, 3-hydroxytetradecenoate, 3-hydroxyhexadecenoate	1,3-Butanediol		[63]
	Polyester of hydroxyalkanoic acids with even carbon number C4, C6, C8, C10, C12	Sodium gluconate		[64]
Pseudomonas sp. GPo1	Unsaturated random copolymer poly(3-hydroxyoctanoate-*co*-3-hydroxyundecenoate)	Mixture of sodium octanoate and undecenoic acid	Fraction of unsaturated units related to the ratio of carbon sources in the medium	[60]
Burkholderia cepacia (formerly *Pseudomonas cepacia*)	3HB-*co*-3H4PE	Gluconate or sucrose		[65]
	Terpolyester 3-HB-*co*-3HV-*co*-2-methyl-3-hydroxybutyric acid	Tiglic acid with or without gluconic acid		[65]

Table 22.3

Overview of PHAs produced by selected bacteria. Data taken from [37]

Bacteria	PHA produced	Substrates	Special feature	Reference
Ralstonia eutropha	PHB, PHBV			[66]
	P3HB-*co*-4HB			[53, 67]
	P3HB-*co*-3HV-*co*-5HV			[68]
	P3HB-*co*-4HB-*co*-3HV			[69]
	P3HB-*co*-3WHV-*co*-4HV			[70]
				[70]
	P4HB pure homopolymer			[71, 72]
Alcaligenes latus	PHB			[73]
	PHBV			[74, 75]
	P3HB-*co*-3Hproprionate			[62]
	P3HB-*co*-4HB			[63]
Alcaligenes eutrophus	P3HB-*co*-3HV			[76]
Rhodospirillum rubrum	Copolymer 3HB+3HV+3H4-pentenoate	4-Pentenoic acid or pentanoic acid	Same product using both substrates	[77]
Chromobacterium violaceum	poly3HV homopolymer	Sodium valerate		[78]
Delftia acidovorans	P(3HB-*co*-4HB) copolymer	1,4-Butanediol		[65]
Methylobacterium	P(3HB-*co*-4HB) copolymer	Methanol + 3-hydroxypropionate		[79]
Comomonas testosterone	P3HB-*co*-Hcaproate			[80]
	P3HB-*co*-Hcaproate-*co*-Hoctanoate		2–4% of comonomer	[80]
Sphaerotilus natans	P3HB-*co*-3HV	Glucose + sodium propionate		[81]

The stereochemistry is very important with respect to biodegradability. In terms of stereoregularity, synthetic PHAs can be made almost identical to the corresponding biopolymers [86]. This resemblance results even in the excellent biodegradability of the material [50]. However, although academically interesting, these synthetic homologues of bacterial PHAs are unlikely ever to be competitive with PHAs produced by fermentation owing to the high lactone monomer costs [5].

22.3.3 Genetic engineering

A novel potential way of synthesizing PHAs calls upon transgenic plants prepared by cloned biosynthetic genes [87]. If this genetic engineering approach were successful and economically feasible, the basic idea would be to use the procedure for the genetic modification of plants to produce polymers through more or less standard agriculture processes, while introducing the same mechanisms as used by bacteria.

The research can be divided into two different routes. The first approach consists in cloning the appropriate genes and transferring them into other organisms. Thus, in 1988, Dennis and co-workers cloned the entire set of genes in *R. eutropha* for the three enzymes involved in the synthesis of PHB from acetyl-CoA [45]. The three genes were clustered in one operon, which was successfully introduced into *E. coli*, and the ensuing association was able to synthesize PHB in large quantities from a wide range of organic compounds. Some recombinant strains of *E. coli* can also produce the HB/HV copolymer [88]. Alternatively, strains containing only the synthase gene can express this protein in sufficiently large quantities for isolation and purification [89]. The purified enzyme can be used for *in vitro* polymerization reactions of various 3- and 4-hydroxyalkanoate-CoA monomers [90], which can have a living character, meaning that the propagating end groups remain active indefinitely and very high molecular weights can be reached [91].

The second approach consists in the successful application of genetic engineering directly to the plants, as reported by Somerville and co-workers in 1992. The reductase and synthase genes of *A. eutrophus* were inserted into a plant, *Arabidopsis thaliana*, which can also produce acetoacetyl-CoA. In this way, the transgenic plant can accumulate PHB granules to approximately 14 per cent of its dry weight [92]. A number of other reports appeared recently, dealing with various aspects of PHA (mainly PHB) production in genetically modified plants [87, 93–95]. Monsanto has recently developed a procedure for obtaining genetically modified plants able to produce small quantities of PHB [96].

To date, however, few reports on the successful production of PHAs have been published, with the exception of a number of studies with transgenic Arabidopsis. Using a variety of chimeric constructs, Steinbuchel and co-workers have determined [97] that the constitutive, chloroplast-localized expression of the β-ketothiolase (*phb*A) gene involved in PHB production, is detrimental to the efficient production of transgenic PHB. The use of either inducible or somatically activated promoters allowed the formation of transgenic PHB-producing potato (*Solanum tuberosum*) and tobacco (*Nicotiana tabacum*) plants, although the amount of PHB formed was still rather low, namely $0.09\,mg\,g^{-1}$ dry weight for potato and $3.2\,mg\,g^{-1}$ dry weight for tobacco [97]. Although very interesting from a scientific point of view, these yields are too low to be considered as a basis for a high volume production of PHA. Nevertheless, this type of research continues based on the concept that the proven genetic engineering way can be developed to an economically applicable biotechnological process, thus contributing, not only to the production of environment-favourable plastics, but also to new perspectives for agricultural development.

22.4 STRUCTURE

The structure of PHAs is based on polyester macromolecules bearing optically active carbon atoms. Two modes are generally described in biological papers, namely medium chain-length PHB (mcl PHB) as the energy reserve supply and hydroxybutyrate cyclic oligomers able to form complexes with other biomacromolecules, for example calcium polyphosphate or proteins acting as ion transfer agents [98].

Poly(3-hydroxybutyrate) is a linear polyester with helical macromolecules. The secondary structure of PHB is specified as left-hand 2_1 helix in a g^+g^+tt conformation, while the structure of oligolides consists of right-hand 3_1 helices [99]. The surface of the $3_1(+)$ helix is covered by methyl groups, leading to the lipophilic nature of the macromolecule. The carbonyl bonds in the $2_1(-)$ helix are placed perpendicularly, while in the $3_1(+)$ counterpart, they are parallel to the helix axis. The latter is the reason for ability to form ionic complexes.

The 3-hydroxyalkanoic acids are all in the [*R*] configuration resulting from the stereospecificity of the enzyme involved in the polymerization step [3]. However, certain portions of *S* units have also been reported [100]. The molecular weight of the simplest poly-3-hydroxybutyrates depends on the bacteria used and the conditions of the synthesis [101], and the way of separation and purification may also have a certain influence. Using some sophisticated procedures, higher molecular weights can be attained, for example an Mn as high as 20 kD was measured for a polymer produced by a recombinant strain of *E. coli* [102].

The formation of hydrogen bonds contributes significantly to the good properties of PHAs. The number of hydrogen bonds formed in semicrystalline polymers may increase with increasing temperature as the crystalline phase progressively melts. For example, when studying isotactic(i) PHB/catechin mixtures, the hydrogen bonding between iPHB and catechin could not be detected by FTIR at room temperature. However, strong hydrogen bonds were confirmed at 190°C, that is above the melting point of iPHB [103]. The strength of the hydrogen bonds is influenced by the tacticity of the polymer chain. An FTIR study, performed by Iriondo *et al.* on blends of poly(4-vynilphenol) (PVPh) and PHB with different tacticity [104, 105], indicated that the hydrogen bonds between PVPh and iPHB were weaker than those between PVPh and atactic(a) PHB [104].

Inoue *et al.* studied by FTIR the hydrogen bonds between catechin and PHB as a function of the latter's tacticity [106] and showed that strong inter-associated hydrogen bonds formed in aPHB/catechin and syndiotactic(s) PHB (sPHB)/catechin blends, while no evidence was found to confirm the existence of such bonds in iPHB/catechin blends [106]. Since the crystallinity of PHB is significantly affected by the tacticity, and the crystalline phase restrains the formation of hydrogen bonds, they also carried out FTIR measurements on the melt at 190°C to separate the effect of tacticity from that of crystallinity. In this case, strong inter-associated hydrogen bonds were detected in iPHB/catechin blends as well as in aPHB/catechin and sPHB/catechin blends [106]. Furthermore, it was found that in the melt, a higher PHB tacticity facilitates the access of the OH group of catechin to the carbonyl group of PHB, and hence the formation of hydrogen bonds.

22.5 ULTIMATE PROPERTIES

22.5.1 General features

Biodegradability is arguably the major advantage of PHAs in terms of their perspective uses and applications. This favourable feature can compete to a certain extent against disadvantages such as higher prices, when compared with conventional plastics. However, a more comprehensive assessment of ultimate properties is crucial when considering any application aimed at everyday commercial products. Table 22.4 gives a comparison of the properties of both PHB and a few PHA copolymers with those of the two most common polyolefins, namely polypropylene and low density polyethylene.

It can be seen that the properties of PHB are rather close to those of polypropylene, outperforming polyethylene in most parameters. The factor of primary negative importance is the low deformation at break, related to low film toughness and unacceptable rigidity and brittleness. The reason for the PHB brittleness arises mainly from the presence of large crystals in the form of spherulites. On the other hand, the high strength and E modulus represent a suitable starting point for possible polymer modifications, since there is a large margin in these strength parameters to increase deformability and toughness.

Copolymers exhibit properties much closer to those of LDPE, but their availability and price still represent a hindrance for these materials to be considered as serious competitors against commodity polyolefins.

Interesting information can be drawn from a comparison of the properties of various commercial biodegradable plastics, as seen in Table 22.5.

Table 22.4

A comparison of the physical properties of PHB and some of its copolymers with those of polypropylene (PP), and low density polyethylene (LDPE). Most data taken from [3]

	PHB	20V[a]	6HA[b]	PP	LDPE
Melting temperature (°C)	175	145	133	176	110
Glass transition temperature (°C)	4	−1	−8	−10	−30
Crystallinity (%)	60	ng	ng	50	50
Density ($g\,cm^{-3}$)	1.25	ng	ng	0.91	0.92
E modulus (Mpa)	3.5	0.8	0.2	1.5	0.2
Tensile strength (Mpa)	0	20	17	38	10
Elongation at break (%)	5	50	680	400	600

[a] Poly(3-hydroxybutyrate-*co*-20 mol% hydroxyvalerate).
[b] Poly(3-hydroxybutyrate-*co*-6 mol% Has), HAs (hydroxyalkanoates) = 3% 3-hydroxydecano-ate, 3% 3-hydroxydodecanoate, <1% 3-hydroxyoctanoate, <1% 5-hydroxy-dodecanoate, ng = negligible.

Table 22.5

A comparison of the physical properties of PHBV with those of other biodegradable plastic

Polymer	PHBV	PLA	PCL	PEA	PBSA	PBAT
Density	1.25	1.25	1.11	107	1.23	1.21
Melting temperature (ºC)	153	152	65	112	114	110–115
Tg (ºC)	5	58	−61	−29	−45	−30
Crystallinity (%)	51	0–1	67	33	41	20–35
Modulus (MPa)	900	2000	190	260	250	52
Elongation at break (%)	15	9	>500	420	>500	>500
Water permeability $g\,m^{-2}$ per day	21	172	177	680	330	550

PHBV – poly-(3-hydroxybutyrate-*co*-3-valerate), Monsanto (Biopol D400G, HV 7%); PLA – poly(lactic acid), Dow-Cargill (Nature Works); PCL – polycaprolactone, Solvay (CAPA 680); PEA – polyesteramide, Bayer (BAK 1095); PBSA – poly(butylene succinate-*co*-adipate), Showa (Bionolle 3000); PBAT – aromatic copolyester, Eastman (Eastar bio 14766).

The density of all these materials is above unity, which may be considered as a certain disadvantage. The melting temperatures are within a reasonable range, except that of PCL, which is a little low for practical applications, considering its possible exposure to sunshine in summer. In terms of the glass transition temperature, these materials may be separated into two groups, one containing just PLA with a T_g well above RT, and the other with T_gs below the temperatures commonly reached in winter in the majority of inhabited countries. Thus, PLA can be considered for applications where common polystyrene is used at present, whereas the other materials can compete mainly with packaging foils made from polyethylene. PHAs are in a special position from this point of view, since, on the one hand, their T_g lies somewhat below, but still close to common RT, so that the material is brittle. On the other hand, it is possible to lower their T_g and thus make the materials ductile by plasticizing them, blending them with miscible additives or by the formation of two-phase systems by blending them with suitable immiscible polymers. The crystallinity of these biodegradable materials lies within a reasonable range, typical of conventional thermoplastics, going from amorphous PLA up to PCL with crystallinity slightly higher than that of LDPE. The modulus is more or less reciprocal to the glass transition temperature with its highest value for brittle PLA and its lowest for PBAT. The values of elongation at break correspond to the T_g being rather high for polymers in a plastic state. From the point of view of PHAs, its low water permeability is certainly an interesting property, suggesting possible applications for packaging.

The material properties of PHAs can be adjusted by varying the HV content, its increase resulting in an increase in the impact strength and a decrease in melting temperature and glass transition [107] (see Figs 22.2 and 22.3), tensile strength [108], crystallinity and water permeability [109]. Different results have been published concerning the mechanical properties of P3HB-*co*-P3HV copolymers [110]. A rather unusual behaviour was reported suggesting that the 3HB and 3HV units are isodimorphous, that is because of their similarity in shape and size, the 3HV units are incorporated into the P(3HB) crystal-lattice [111]. It follows that the properties of P(3HB-*co*-3HV) are not significantly improved in comparison with the P(3HB) homopolymer. Copolymers with improved properties can be prepared via the copolymerization of 3HB with longer chain hydroxyalkanoic acids, which form a separate crystalline lattice or do not crystallize at all. Examples of this type of copolymers, showing exclusion of the longer chain comonomer units, are the copolymers of 3HB and 3-hydroxyhexanoate (3HH) [112].

22.5.2 Glass transition, melting and crystallization

PHB synthesized by bacteria and separated by standard procedures is a semicrystalline polymer with a rather high crystallinity, which can reach 80 per cent. It was found surprising that bacteria could produce crystalline polymers and even more surprising that they were able to use it as a feedstock. Until the mid-1880s, the prevailing belief was that PHA *in vivo* was indeed a crystalline solid [113, 114]. The first doubts about this morphology of P3HB were raised because of the observation that a short centrifugation resulted in an irreversible loss of intracellular degradability. The final solution regarding these inconsistencies was obtained from solution-state NMR spectra which showed [115] that poly(3-hydroxybutyrate) *in vivo* is not crystalline, but rather a completely amorphous material. It was later demonstrated that centrifugation gave rise to completely different features in the NMR spectra and the simultaneous appearance of X-ray powder diffraction patterns, typical of crystalline P3HB [116]. The amorphous character of *in vivo* P3HB is an obvious and fully acceptable idea, but the question is immediately raised regarding the mechanism triggering its crystallization when the inclusions are isolated. The existence of *in vivo* plasticizers or nucleation inhibitors was put forward as an explanation [3], but no proof has yet been found for this proposal.

The crystalline structure of PHB is orthorhombic with dimensions of the basic crystalline cell $a = 5.76$ Å, $b = 13.20$ Å, $c = 5.96$ Å. A partially planar zig-zag structure can be formed as a result of the mechanical uniaxial load from the amorphous phase between lamellae [117, 118]. PHB forms extremely thin lamellar crystals, with a thickness comprised between 4 and 7 nm and a prevailing size of 5 nm [98].

A number of data are available of the glass transition and melting temperatures of P3HB and its copolymers with P3HV. The equilibrium melting point T_m^o is 470 ± 2 K, according to Barham *et al.* [119], although the former author in a later paper reported a broad range of T_m^o, changing with molecular weight, from 352 K (Mw ~ 20 000) up to the previously reported 471 K (Mw ~ 300 000) [120]. The melting enthalpy for the 100 per cent crystalline PHB is 146 J g^{-1} [119]. The real melting temperature, as measured by optical observation, does vary significantly with molecular weight, as seen in Fig. 22.2, which shows that the T_m of oligomeric PHB increases almost linearly in the Mn range of 350 to around 700. The curve becomes less steep and the linearity disappears. Finally, the T_m

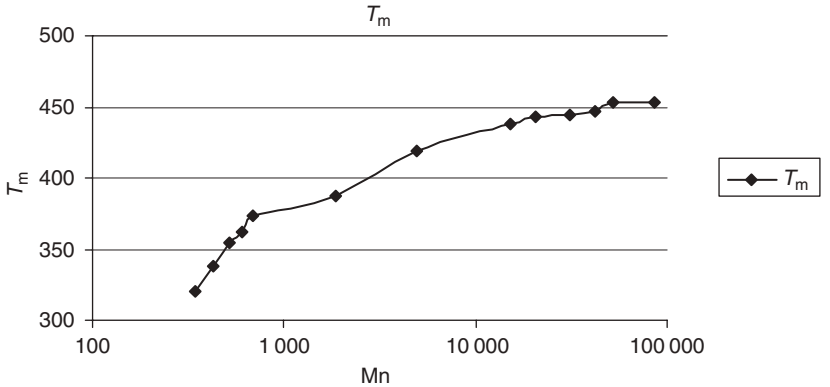


Figure 22.2 Melting temperature of P3HB as a function of its molecular weight. (Data taken from [121], original source [122].)

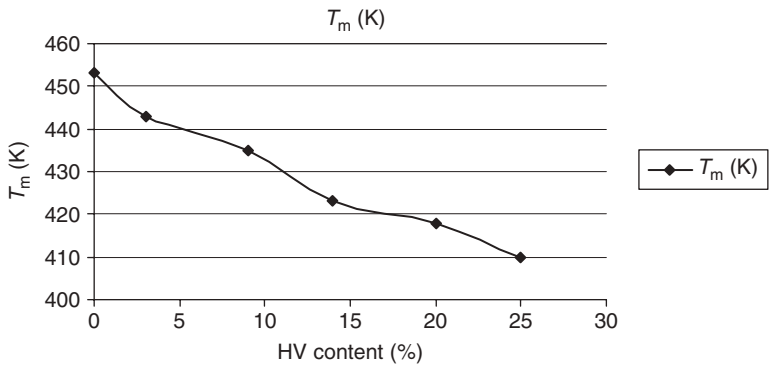


Figure 22.3 Melting temperature variation as a function of the comonomer content for poly-3-hydroxybutyrate-*co*-3-hydroxyvalerate copolymers. (Data from [121], original source [44].)

of polymers with molecular weights above 50 000 levels off at a value of 453 K. A practically linear decrease in T_m was reported for a set of P3HB-3HV copolymers with rising HV contents, as seen in Fig. 22.3.

Due to its low nucleation density, a small number of large, rather imperfect, crystallites grow in PHB, affecting its mechanical properties, especially the elongation at break and the brittleness of moulded products and films. This effect can be eliminated to a certain extent by selecting the crystallization conditions, especially the crystallization temperature, since the morphology of the crystalline region depends significantly on this parameter [119]. The addition of nucleating agents and suitable post-treatments after extrusion or casting can also lead to much improved properties [40].

PHB copolymers with higher alkanoates usually exhibit lower T_g values than that of the corresponding homopolymer. The poly([*R*]-3-hydroxybutyrate-*co*-5%-[*R*]-3-hydroxyhexanoate) (P(3HB-*co*-5%-3HH)) with $M_w = 0.82 \times 10^6$ and DPI = 2.2, and poly([*R*]-3-hydroxybutyrate-*co*-12%-[*R*]-3-hydroxyhexanoate) (P(3HB-*co*-12 per cent-3HH)) with $M_w = 0.93 \times 10^6$ and DPI = 2.2 were reported to have T_gs of 277 and 270 K, respectively, with the corresponding T_m at 417 and 385 K [110]. It is interesting that the homopolymer of 4-hydroxybutyric acid (P(4HB)) showed much lower values for both its glass transition (∼−50°C) and melting (∼60°C) temperatures, as measured by calorimetric analysis [67, 82].

22.5.3 Ageing of PHB by physical processes

It is known that PHB ages upon storing with an increase in its Young's modulus accompanied by a decrease in its elongation at break which translates into a pronounced increase in brittleness of the material within a few

months after thermal processing [123]. This phenomenon is only partially understood and has been rationalized by two alternative explanations. The first is termed physical ageing and is based on the relaxation process usually observed in amorphous polymers. When the material is cooled from the melt to glassy state, the amorphous domains do not reach their equilibrium free volume. If the material is stored close to its T_g, the chains reorganize slowly and the corresponding free volume decrease occurs. Thus, the reason for the extended effect in PHB arises from the fact that its T_g is only a little below room temperature. Reorganization proceeds extremely slowly (within few months) compared to other polymers, but the movements are not completely frozen as they would be if the storing temperature were well below T_g, as is the case for most amorphous polymers.

It is believed that physical ageing is responsible for the pronounced brittleness of PHB [124–126]. A decrease in the maximal intensity of the loss factor $tg\delta$ around T_g with storage confirmed the occurrence of a relaxation process [124]. Hurrell and Cameron provided an identical conclusion by investigating the process by SAXS and proved that the microstructural changes observed do not fully correspond with the changes in mechanical properties [126].

The other explanation of the process consists in suggesting a secondary crystallization proceeding very slowly because of the low chain mobility associated with the temperature being close to T_g. In this process, the lamellae become progressively more perfect, leading to a reduction of the interphase between amorphous and crystalline domains. Some of the chains in the amorphous phase are more stretched and this introduces a tension within it [127]. This progressive crystallization produces a growth of 7 per cent crystallinity within 8 days from the initial value 56 per cent, measured immediately after the thermal processing. Moreover, storing at temperatures higher than RT leads to an increase in the ultimate crystallinity, a process which is more or less completed after a month with a crystallinity of about 65 per cent and an end to the evolution of the mechanical properties [125]. Other authors, however, have observed that the crystallinity only increases during the first few hours after cooling the melt [128].

A detailed investigation of the ageing behaviour of PHB copolymers containing 8 or 12 per cent of valerate units has been reported [129]. Both extruded and compression moulded samples were investigated by DSC, DMTA, dielectric spectroscopy, and thermally stimulated discharge. Besides changes in the amorphous and crystalline phases, the authors considered the importance of the interphase region on the ageing process, consisting in relaxation above T_g due to morphological reorganization in the interphase [129]. This opinion is supported by fracture mechanics data indicating that the ageing process does not consist of a simple embrittlement, but rather of a reduction in the energy dissipating properties of the material, accompanied by the ability to survive high stress levels [130]. From this point of view, the changes in FTIR spectra of PHB during storage may be of interest. Two IR peaks have been found [131] to be sensitive to storage, namely at $1\,685\,cm^{-1}$ and $3\,435\,cm^{-1}$, corresponding to carbonyl and hydroxyl stretching, respectively. Both peaks grow during storage with no levelling off after 14 days [131], indicating that some interactions between the functional groups of PHB may result from storage. It is not clear to what extent these interactions are related to the physical ageing. Nevertheless, it is worth considering the importance of these effects in addition to the changes in the crystalline structure, especially in the interphase region.

22.5.4 Solubility and plasticization

The solubility of PHB in various solvents (see Table 22.6) is of importance, not only from a scientific point of view, but also in terms of the selection of a suitable solvent for the preparation and purification of the polymer from bacteria.

Although generally PHB is considered to be a hydrophobic material, it is, in fact, slightly hydrophilic [132, 133], that is the ratio between its hydrophobic (dispersive) and hydrophilic (polar and electrostatic) interactions depends, to a certain extent, on the morphology and degree of orientation of the anisotropic units. While for isotropic structures, the presence of imperfect crystallites may result in the formation of sites for water absorption (especially accessible carbonyl moieties), in anisotropic materials (*e.g.* fibres), the sorption capacity is substantially lower. On the other hand, the sorption of some organic solvents, such as acetone, does not depend on the PHB morphology [134]. Thus, the small molecules of water can act as a kind of structural probe, while the larger acetone molecules do not recognize the difference between the iso- and aniso-structures of PHB. The linear dependence of the diffusion coefficient on the sorbed water concentration in PHB indicates that water acts as a plasticizer for this matrix. Similar effects have been obtained for various organic solvents. Limiting diffusion coefficient values increase in the order benzene = ethanol < hexane < acetone.

Table 22.6

Solubility of PHB in various solvents [83, 121]

Good	Partial	Not soluble
Chloroform	Dioxane	Water
Dichloromethane1	Toluene	Methanol
1,2,2-Tetrachloroethane	Pyridine	Ethanol
Ethylene carbonate	Benzene	Isopropanol
Propylene carbonate	Xylene	Cyclohexanol
Acetic anhydride		Diethyl ether
N,*N*-dimethylformamide		*n*-Hexane
Ethylacetoacetate		Cyclohexanone
Acetic acid		Ethyl acetate
Higher alcohols (C > 3)		Ethyl methyl ketone
2,2,2-Trifluoro ethanol		Tetrahydrofurane
		Butyl acetate
		Valeric acid
		Diluted mineral acids

Butyl chloride is reported as a solvent for the synthetic PHB at 13°C [83].

From a practical point of view, chloroform or dichloromethane is commonly used for the extraction of PHB or its copolymers from the bacteria, for example in [124], a preparation of a bacterial P(3HB-*co*-4HB) copolymer was described based on the fermentation of *D. acidovorans* from 1,4-butanediol [82], where the product was extracted from the lyophilized cells with hot chloroform and purified by reprecipitation with hexane. Then, the precipitate was fractionated in boiling acetone, and the soluble part taken as the P(3HB-*co*-4HB) sample containing 7 mol% of 3HB units.

The solubility of PHAs differs depending on their composition, for example after a PHA synthesis by *Pseudomonas sp*., the acetone-insoluble fraction contained the poly-3-hydroxybutyrate homopolymer, while the acetone-soluble fraction was reported to consist of seven different 3HA units ranging from C4 to C12 [64].

Another reason for investigating solubility and/or miscibility with various liquids of low molecular size and relatively high boiling points, is their possible application as plasticizers, as reported in some studies [135, 136]. Soybean oil (SO), epoxidized soybean oil (ESO), dibutyl phthalate (DBP) and triethyl citrate (TEC) have also been described as plasticizers [137]. PHBV blends containing 20 wt% of plasticizer were prepared by evaporating the solvent from the blend solutions. The compatibility of the plasticizers was then examined using DSC and SEM and their efficiency estimated according to the T_g decrease. DPB and TEC were found to be more effective than SO and ESO. The extent of the decrease in the T_g induced by plasticizers varies, leading, in some cases, to an increase in the elongation at break, as in the case of the addition of around 32 wt% of oxypropyl glycerol, dibutyl sebacate or dioctyl sebacate to PHB, which resulted in an elongation at break of more than 250 per cent [138].

It has to be stressed that some plasticizers which are commonly and successfully used with other polymers, may act as prodegradants for the thermal degradation of PHAs. Glycerol has been reported to act in such a way, while glycerol triacetate (triacetin), although effective as a PHB plasticizer, was found to be inert as a prodegradant [139]. Acetyl tributyl citrate was also examined in this context [140].

Obviously, the biodegradability of a given plasticizer is a fundamental prerequisite for its use in biodegradable plastics such as PHAs, as are any possible health hazards associated with it or with its decomposition products. Both aspects have been taken into account in studies involving additives such as citrate-based plasticizers [141, 142], or the new benzoate plasticizer, Benzoflex® 2888, a blend of diethylene-, triethylene-, and dipropyleneglycol dibenzoate [143].

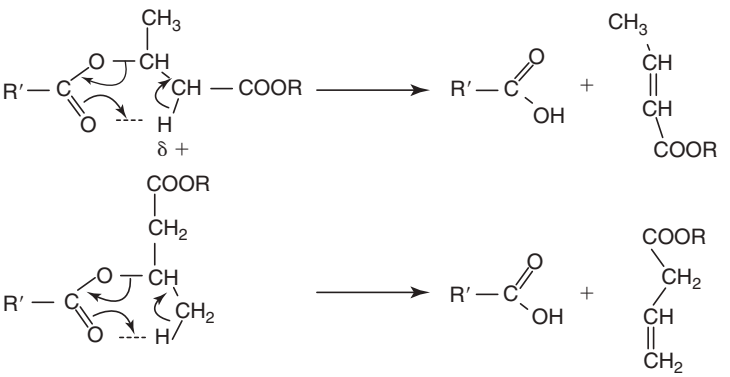
22.6 THERMAL AND HYDROLYTIC DEGRADATION

The very high susceptibility of PHB and also of other PHAs, to thermal degradation, represents a serious problem, especially with respect to processing. Considering its melting point, the processing temperature of PHB should be at least 190°C. At this temperature, thermal degradation proceeds rapidly, so that it is impossible to avoid certain, sometimes substantial, decomposition consisting of a decrease in molecular weight and, consequently, a

Table 22.7

GPC molecular weights after the thermal treatment (1, 10 and 80 min) of PHB and PHB with 20 wt% of glycerol triacetate (TAC) and glycerol plasticizers, as a function of the annealing temperature. Data taken from [139]

Sample	Annealing		MW $g\,mol^{-1}$
	°C	Minutes	
PHB	170	1	270 000
	180	10	97 000
	200	10	29 000
	220	10	4 500
PHB/TAC	170	1	240 000
	200	10	30 000
	220	10	6 000
PHB/glycerol	170	1	170 000
	200	10	9 000
PHB	190	80	6 000
PHB/TAC	190	80	9 000
PHB/glycerol	190	80	<3 000



Scheme 22.2 Thermal *cis*-elimination in polyesters.

pronounced effect on the mechanical and other ultimate properties. The extent of this degradation is illustrated by the changes in molecular weight after annealing at various temperatures shown in Table 22.7.

The thermal degradation of polyesters is generally described either by a *cis*-elimination or a transesterification. The former process dominates with ester moieties with acidic C═H bonds due to activation via the carboxyl groups, as shown in Scheme 22.2, with the formation of polymer fragments [144]. *Cis*-elimination is a non-catalyzed process, almost uninfluenced by the presence of additives or impurities, and its rate is similar in the liquid or gas phase.

P(3HB) with three carbon atoms in the main-chain monomer unit, degrades by the *cis*-elimination reaction, releasing crotonic acid and linear oligomers of 3HB containing crotonyl chain ends as volatile products [145]. However, the changes in molecular weight indicate that more complex side reactions occur. Gel permeation chromatography (GPC) measurements of PHB heated at various temperatures, as a function of time, revealed that the molecular weight initially decreased, but then increased slightly before continuing to decline [146]. This effect was attributed to the polycondensation of the initial hydroxyl and carboxyl groups formed by the elimination process. Severe degradation conditions (*e.g.* up to 300°C under vacuum) lead to the formation of crotonic and isocrotonic acids and the dimer, trimer and tetramer of PHB [147].

Transesterification must also be considered when blends of PHB with other polymers are investigated, especially if they contain hydroxyl, carboxyl or other reactive moieties. A significant decrease in the activation energy of the thermal decomposition of PHB was observed if PMMA was present as a second component in the blend, although the changes were not attributed to any particular PHB/PMMA interaction, whereas only marginal changes were registered if PHB was mixed with polypropylene, as revealed by thermogravimetry [148].

The presence of various additives alters the thermal degradation kinetics with many species acting as prodegradants. Aluminium compounds and fumed silica have been reported to have a slight stabilizing effect, followed by a prodegradant activity [2]. Other inorganic compounds have been shown to be prodegradants, as revealed by dynamic TG experiments [149] and the same effect was observed for impurities present in technical PHB when compared with a carefully purified sample [150].

As expected, degradation has a negative influence on the mechanical properties of PHB [151]. From this point of view, it is important to estimate the effect of plasticizers on the thermal degradation. As shown in Table 22.7, glycerol triacetate does not play any significant role, while glycerol has a detrimental effect on the molecular weight during processing [139], which is attributed to a transesterification reaction in which the hydroxyl groups of glycerol play a crucial role.

Given the high rate of thermal degradation of PHB, its processing by injection moulding or extrusion must be carried out with the lowest possible temperature and residence time [154, 155].

Stabilization of PHB by conventional methods is almost impossible. Antioxidants are ineffective, so are other additives, such as acid acceptors, moisture adsorbers, chelates, blockers of hydroxyl and carboxyl groups (acetic anhydride or diazomethane, respectively). Some improvement was achieved by the addition of sulphur and additives releasing sulphur dioxide. The presence of certain inorganic additives (CaO, PbO, PbO_2, Al_2O_3, ZnO) leads to destabilization according to the acidity of the additive. On the other hand, more stable products are formed by the reaction with MgO, so that the decomposition reaction increases to about 670 K, that is about 100 K higher compared with PHB alone [149]. The presence of aluminium complexes results in crosslinking, which to a certain extent, heals the effect of the thermal main-chain cleavage [2].

Since the thermal stabilization of PHB has not been satisfactorily solved yet, it is necessary to look for processing procedures operating at lower temperatures. From this point of view, the addition of plasticizers is definitely an option, since it leads to a decrease in the melting temperature of 15–20°C, although this requires rather high amounts of the plasticizer, up to 30 wt%.

It is interesting that the presence of a second monomer in PHA copolymers has a certain stabilizing effect in their thermal degradation, as revealed by thermogravimetry for PHB and its copolymers with hydroxyvalerate and hydroxyhexanoate [156]. Moreover, in the case of the PHBV copolymer, two peaks were observed in the thermograms, indicating that the two different monomers evaporated at different temperatures [157]. The thermal degradation of various P(3HB-*co*-4HB) copolymers containing between 11 and 82 mol% of 4HB units, was reported to occur by a random chain scission process involving *cis*-elimination below 200°C [158]. On the other hand, Abate *et al.* reported that the main degradation mechanism of P(3HB-*co*-4HB) containing 97 mol% of 4HB units is an unzipping reaction starting at the chain ends [227]. They also suggested that the existence of 3HB unit would cause a competition between their *cis*-elimination and the unzipping reaction [gi]. Two processes were also identified during the non-isothermal degradation of P(4HB) at temperatures below and above 350°C and the major volatile product was γ-butyrolactone, regardless of the degradation temperature, indicating that its selective formation via an unzipping reaction predominated at temperatures below 300°C. At temperatures above 300°C, both the *cis*-elimination reaction of 4HB units and the formation of cyclic macromolecules of P(4HB) via intramolecular transesterification took place in addition to the unzipping reaction [158, 159].

As mentioned above, PHB takes up minor quantities of water upon storage [152], that is, about 0.2 wt%. Despite this modest moisture content, the possibility of polymer degradation by hydrolysis must be taken into account since PHAs degrade in water at room temperature, albeit at a very low rate. This hydrolytic degradation is strongly accelerated at higher temperatures or in an alkaline medium [153].

22.7 MODIFICATION

The modification of PHAs is aimed at improving certain ultimate properties. Since the price of copolymers is still higher compared to P3HB, the most interesting approach seems to be to modify the simplest homologue, that is P3HB, to prepare PHA-based biodegradable materials with good mechanical and other properties and acceptable prices. An alternative route consists in synthesizing various copolymers, especially P3HB-3HV, with the aim of developing a process producing them at a lower price. However, the modification to change properties is interesting even in the case of copolymers.

Generally, the methods of modification are based on chemical (changes of chemical structure, *e.g.* grafting, crosslinking, transesterification) or physical procedures (mixing with low or high molecular additives, optimization of crystallization) and these two approaches are discussed below.

22.7.1 Chemical methods

As briefly discussed above in the context of ultimate properties, the introduction of comonomer, especially hydroxyvalerate or hydroxyhexanoate, leads to higher elongation and toughness [5] and a modest improvement of thermal stability [156], accompanied by a decrease in modulus and, in many cases, of strength.

Transesterification with glycol is frequently used to prepare telechelic diols of PHB and P(HB-*co*-HV) in the presence of dibutyl tin dilaurate as catalyst [160]. The ensuing oligomers with well-defined end groups are suitable for the synthesis of block copolymers [161, 162], whose mechanical properties depend on the ratio of hard and soft segments. The synthesis of poly(H3B-*co*-caprolactone, CL) uses this principle, where the transesterification of the homopolymers, catalyzed by acids, results in the formation of random or microblock copolymers [163]. With rising CL content, a decrease in T_g is observed from 2 down to $-42°C$, for CL contents of 0 and 72 per cent, respectively.

Crosslinking is a basic method for preparing thermosets, but it is also commonly used to modify a number of elastomers or thermoplasts. Polyhydroxyalkanoates can be crosslinked if the process is initiated by the thermal decomposition of peroxides, although the crosslinking efficiency is not too high, so that a crosslinking coagent is recommended together with an initiator. An increase in viscosity by a factor of 20 was observed when PHB was treated with a mixture of dicumyl peroxide and triallyl cyanurate as a coagent [2]. Crosslinking does not affect the chain cleavage resulting from thermal degradation, but it eliminates, and sometimes overrules, the decrease of molecular weight due to degradation. During the thermal degradation of PHB via *cis*-elimination, crotonyl and carboxyl groups are formed, and the former can be expected to react with free radicals formed by the thermal decomposition of the peroxide to form crossbonds. Initiation by irradiation is claimed to be ineffective, since it leads to a pronounced degradation of the PHA chains [164]. However, this depends on the actual composition of the specific PHA, since successful crosslinking was reported using electron beam (EB) irradiation of unsaturated PHAs [165], and the ensuing network had elastomeric properties and was still completely biodegradable. A rather successful crosslinking was also reported using initiation by gamma irradiation, especially if the process was carried out under nitrogen instead of air [166]. In this case, the copolymer was poly(3-hydroxyoctanoate-*co*-undecenoate), for which a much higher efficiency can be expected, compared to PHB or PHBV, not only because of the presence of unsaturations, but also as a result of the long saturated carbon chains, which can be sites of hydrogen abstraction forming free radicals ready to recombine and crosslink. Crosslinking results in a modest increase in T_g and an improved shape stability at temperatures above T_m.

Crosslinking has been suggested to be a reason for the improved thermal stability of PHB/poly (glycidyl methacrylate) blends. In this case crossbonds are formed via the reaction of the epoxy groups of PGMA with the carboxyls of PHB [167].

Free-radical initiated reactions can also be used, besides crosslinking, for other modifications. The thermal decomposition of benzoyl peroxide was successfully used to initiate the grafting of maleic anhydride on PHB which was reported to produce a material with increased thermal stability and biodegradability, the latter attributed to the appended maleic moieties [168, 169].

PHAs containing specific functional groups (halogen, nitrile, double bond) can be modified, not only by crosslinking via peroxides or sulphur, but also by more sophisticated procedures. Thus double bonds can be transformed into epoxy moieties, which can be further used for a number of modifications, including crosslinking [170]. The grafting of poly(3-hydroxyoctanoate-*co*-3-hydroxyundecenoate) was shown to proceed also via the oxidation of the pendant double bonds into carboxyls and the esterification of these side moieties with poly(ethylene glycol). This process enhanced the hydrophilic character of the modified polymers, making them soluble in polar solvents, such as alcohols and water/acetone mixtures [171].

22.7.2 Physical modification

As already discussed above, the use of plasticizers produces a decrease in the processing temperature and hence a more convenient processing regime, resulting from the decrease in the melting temperature [2]. Some losses in strength and modulus must be accepted in this context, depending on the amount of plasticizer added.

A hot rolling treatment has been shown to substantially improve the ductility of PHB. It is proposed that rolling results in the healing of cracks present inside the spherulites leading to more ductile films [172]. A more sophisticated approach seems to be the solid-state processing of PHB powders, which prevents thermal degradation, since

the extrusion proceeds well below the polymer melting temperature. The improved mechanical properties were attributed in this case to structural differences at the molecular and supramolecular levels [173]. The changes are apparently similar to those induced by hot rolling; but much higher due to the higher stress.

An interesting and rather simple option for toughness improvement consists in annealing the material at or above 120°C [125]. The elongation at break was found to increase up to 30 per cent [127], with values of up to 60 per cent in individual cases [125]. A change in lamellar morphology during annealing was suggested as an explanation of this effect, resulting in a substantial increase in the mobile phase, as indicated by the area under the $t_g\delta$ peak measured by DMTA. The reason for the morphology changes can be rationalized by the fact that the annealing could be considered as if crystallization proceeded from the melt at a much lower rate.

In conclusion, these types of physical modifications can improve significantly the mechanical properties of PHB, although the extent of these changes is insufficient in terms of its long-term properties.

Blending with other polymers and mixing with solid isotropic or anisotropic particles are efficient ways to produce new materials with highly modified properties using physical modification principles. This topic falls somewhat outside the scope of this chapter, but enough data can be found in the recent literature [128, 174]. Hence, only a few remarks will be given here.

Blends of various PHAs or PHAs with other biodegradable polymers are commonly used for adjusting the properties of the final materials, while maintaining their biodegradability. The blends of two different PHAs are usually immiscible [175], at least in the crystalline state [176], even in the case of a blend of isotactic bacterial PHB and synthetic atactic PHB, which is miscible only in a limited concentration range [177].

PHA blends with poly(vinyl alcohol) (PVA) are interesting materials whose miscibility depends on the composition and the PVA tacticity [178]. PHB is claimed to be miscible with polyethylene glycol [179] and with poly D-lactide [180]; its miscibility with polyethylene oxide depends on the blend composition [181]. Immiscible, but well-compatible blends of PHB, were prepared with poly(butylene succinate-*co*-butylene adipate) and poly(butylene succinate-*co*-caprolactone) [182].

Important materials have been developed based on blends of PHAs with PCL. These blends are immiscible and mainly incompatible [183], but compatibilization can be reached by the addition of poly(HB-*co*-CL) copolymers [184], or by crosslinking [185].

Unlike blends with biodegradable polymers, where the main purpose is to prepare biodegradable materials with modified properties, blends with non-biodegradable polymers are prepared as materials containing a polymer from renewable resources, aiming at attaining certain unique properties, for example an improved barrier behaviour. Examples of such PHB blends include the use of polyvinyl acetate [186, 187], polymethyl methacrylate [188], polyvinyl phenol (miscible blends due to hydrogen-specific interactions) [105] and various rubbers [189], including epoxidized natural rubber [190]. Miscible blends of PHB with poly(vinylidene chloride-*co*-acrylonitrile) [191] and epichlorhydrin [192] have also been reported.

22.8 PROCESSING

The processing of PHAs depends very much on the particular grade of the polymer used. These considerations apply essentially to PHB and perhaps PHBV, since the other PHAs are still in the development stage and hence their processing industrial technology is not yet a concrete issue.

The melt processing of PHB can be performed using conventional technologies; however, it is complicated by two factors. The first problem is the low viscosity of its melt and the low melt elasticity, requiring rather precise conditions for extrusion or injection moulding. The second problem is the low thermal stability of PHAs in general and of PHB in particular, as discussed above, which gives rise, during melt processing, to a substantial decrease in molecular weight leading to both worse ultimate properties and a further decrease in melt viscosity. The extrusion of PHB, recommended for Biomer, Germany, consists in melting the material in the first section of the equipment and a gradual temperature decrease in the next zones. The temperatures for Biomer grade P226 decrease from 185°C in zone 1, down to 150°C in zone 4 and 145°C in the die. For other grades (Biomer P209 and P249), temperatures are lower by 5–10°C.

Processing at lower temperatures can be achieved by the addition of plasticizers or by copolymerization with higher PHAs, as already discussed. In both cases, the ultimate properties change, so that a compromise must be found between an acceptable extent of thermal degradation and the desired properties.

22.8.1 Special modes of processing

Special procedures for PHB processing are applied both to avoid or decrease thermal degradation, and to produce unconventional products. In some cases, the two targets overlap, so that for certain special products unconventional processing is used. The three most relevant applications of these special processes are briefly discussed.

22.8.1.1 Solid-state processing

This technique was recently described in detail by Radusch in his review [193], and its principles are also applicable to other PHAs. The process consists in sintering powders at very high pressures and elevated temperatures, but below their melting point, and involves a two-step solid-state transformation [194, 195]. Deformation degrees expressed as draw ratios (DRs) 1.5 and 2.75 were achieved, depending on the processing conditions. Together with this increase in DR, the density rose as well with an increase in the processing temperature, as seen in Table 22.8, in which data for melt-processed PHB are shown for the sake of comparison.

After solid-state processing, a very different crystalline structure was found, compared to melt-processed PHB, as revealed by WAXS and, in spite of a certain orientation due to solid-state processing, a slight decrease of crystallinity was also reported. The mechanical properties of the solid-state extruded PHB depends substantially on the initial molecular weight of the polymer, as well as on the processing temperature, as seen in Tables 22.9 and 22.10.

Other properties are also significantly affected by solid-state processing, compared to the melt counterpart, viz. dynamic mechanical properties, as well as relaxation behaviour, for example creep [193].

Table 22.8

Changes in draw ratio and density of both solid-state extruded and melt-processed PHB with temperature. Data taken from [193]

Temperature (°C)	25	70	100	120	Melt
Draw ratio, λ/λ_0	1.6	1.8	2.1	2.7	~1.0
Density	1.11	1.24	1.25	1.25	1.25

Table 22.9

Dependence of tensile stress and elongation at break on the initial molecular weight of solid-state extruded PHB. Data taken from [193]

MW ($kg\,mol^{-1}$)	171	350	500	1 000
Tensile strength at break (MPa)	15	38	48	54
Elongation at break (%)	1.0	6.6	7.2	9.1

Table 22.10

Dependence of tensile stress and elongation at break on processing temperature of solid-state extruded PHB. Data taken from [193]

Temperature (°C)	70	100	120	185	185[a]
Tensile strength at break (MPa)	35	48	57	10	39
Elongation at break (%)	2.1	9.2	18.0	0.5	2.4

[a] Melt processed material.

22.8.1.2 Foaming

Biodegradable foams represent interesting products. Depending on their structure and properties, they can be used for simple impact-damping fillings of packages, for transport of brittle products, as well as for more sophisticated applications, mostly based on large surface areas, such as filtering, absorption of various species or drug release. Biodegradability is required for easy waste disposal in the former case, or for performance improvement in the latter.

The present industrial applications of PHB-, or more generally, PHA-based foams, are modest because their preparation is not easy, mainly due to problems of thermal degradation, which are particularly serious in this context, since they make foaming difficult and produce cell collapse after the application of the foaming agents because of the low viscosity of the material.

Few examples of PHB foaming are to be found in the literature. In one of these, PHB was mixed with polyvinyl alcohol and starch, to prepare a composite, for which an optimal viscosity could be attained. Azodicarbon amide was used as the foaming agent. A biodegradability study revealed a positive effect of the larger surface on the rate of biodegradation in foamed products, compared with bulk materials with the same composition [196].

The development of foams for medical purposes was described by Radusch [193]. To eliminate thermal degradation, as well as to avoid the problems associated with the low melt viscosity, a process was developed based on solvent casting and subsequent particle leaching. Well-defined porous structures can be formed in such a way [197–200], for example, PHB was dissolved in chloroform, the ensuing solution was mixed with water-soluble particles like NaCl, films were then prepared by solvent casting and evaporation and, subsequently, the salt particles were washed out with water. Various foamed structures were thus prepared, depending on the particle size and amount, as well as other parameters, such as the PHB type and its concentration in the chloroform solution.

22.8.1.3 Fibres

The preparation of fibres based on PHA is not easy. Usually, exact procedures have to be developed and small deviations from the optimal process may be detrimental to spinning, drawing or to satisfactory final properties of the fibres. Melt spinning seems to be the simplest, although not easiest, way for PHA fibre preparation. For a delicate procedure such as spinning and subsequent drawing, the general processing problems are quite pronounced. Gel spinning was also described in the literature and reported to give fibres with a higher DR and improved mechanical properties, compared to PHB fibres made by melt spinning.

Several methods for both melt and gel spinning have been developed, differing in certain details of the process. A schematic description of the basic steps of these various procedures is given in Table 22.11.

The basic requirements for melt spinning/drawing seem to be the presence of nucleating agents, for example boron nitride [201], on the one hand and the drawing, or at least pre-drawing, while the material has not yet developed a fully crystalline structure, on the other hand. The drawability depends on the drawing temperature to a certain extent, producing the highest DR (about 5) at 120°C. Surprisingly, the drawn materials exhibit, in some cases, a rubber-like elastic behaviour, indicating that drawing leads to changes similar to cold rolling.

The surface of melt-spun fibres consists of many large spherulites, as indicated by SEM observations [210]. After drawing to DR = 6, the fibres have a fibrillar structure and their surface is fairly smooth. Annealing under tension leads to the formation of a more perfect fibrillar structure in the core, and this effect seems to be increasing with a rising tension load during annealing [210]. A study aimed at improving high speed melt spinning and spin drawing was reported by Schmack *et al.* [211]. A spinning line, consisting of an extruder, spinning pump, heated godets and two winders, enabled reaching speeds in the range 2 000–6 000 m min^{-1}.

22.9 APPLICATIONS

No high volume applications of PHAs exist; at present there are only trials and small pilot productions. Two perspective areas of applications are considered here, namely health care and packaging. In the field of medical and pharmaceutical applications, the hydrolytic degradation of PHAs is required as the main degradation mechanism, while in environmental applications, enzymatic degradation mechanisms (biodegradation) are relevant.

Isotropic PHB foils possess excellent barrier properties against gas permeation. This is the rationale for potential applications in packaging, especially for food. The substitution of polyethylene terephthalate (PET) for bottle production seems to be especially attractive, considering the volume of PET waste. Another option consists in the modification of paper since paper/poly(HB-*co*-HV) foils are completely biodegradable, unlike paper coated with conventional foils [212]. The simplest application is in containers, plastic bags and foils, commonly produced

Table 22.11

Procedures for the preparation of PHB fibres

Process steps	Remarks	Reference
Melt spinning		[201]
Melt spinning followed by a pre-orientation	Gordeyev *et al.*, 1977	
Melt quenching below T_g Subsequent drawing		[202, 203]
Melt spinning		
Drawing of melt spun PHB immediately afterwards	Pre-oriented material	
Drawing to high draw ratios	Pre-oriented fibres can be drawn after a few weeks of storing at RT	[201]
Dissolve PHB in chloroform	Molecular weight about 300 000 and T_m = 180°C	[204]
Filtrate the solution before spinning to remove impurities, as well as high molecular weight portions		
Spinning and pre-drawing (DR = 2)	Extruder heated in four regions between 170°C and 182°C	
Extruding	Filaments about 0.3 mm in diameter	[117]
Drawn at 110°C immediately after melt spinning	Maximum DR achieved = 6	
Gel spinning		
Dissolving the PHB in 1,2-dichloroethane	PHB concentration as high as possible	[205]
Solid gel prepared by evaporation of part of the solvent		
Gel extrudable at about 170°C		
Consequently processed in three stages		
Winding the fibre on a speed-controlled drum	Preconditioning stage	
Continuous hot drawing between two rollers at 120°C	Total DR around 10	
Fibres stretched at RT to 180%Fixed and annealed at 150°C for 1 h	DR = 1.8 of the length after second step	
Other procedures		
Solution-cast high molecular weight films stretching to DR higher than 6	Silicon oil bath at 160°C	[206]
Annealing	To avoid brittleness increase due to storing	
Centrifugation – spinning	Pores with diameter in the range 1–15 μm	[207]
PHB-co-higher PHA copolymers		
Solvent – cast films	PHB-hydroxyhexanoate copolymer	[110]
Melted in a hot press and subsequently quenched in ice water	More or less amorphous films	
Oriented by cold drawing	DR 2–5	
Annealed	Various temperatures, 23–140°C	
Blends		
Solvent casting	Poly(L-lactic acid) and PHB	[208]
Uniaxial drawing	At 2°C for PHB-rich blends (close to PHB's T_g) or 60°C for PLLA-rich blends	
Solvent casting	PHB-*co*-hydroxyvalerate and polyalcohols	[209]

from polyolefins. The first commercial product of this type was biodegradable bottles for shampoo produced by the German company Wella AG [213]. However, its volume was marginal, main reason being the high price of PHAs. Thus, the competition with polyolefins in the near future does not look too optimistic for PHAs. Perhaps a better chance can be seen in the partial substitution of PET bottles, where the difference in the price of the basic materials is less dramatic, although in this case, many technological details have to be solved, in particular concerning the low thermal stability and melt viscosity of PHAs.

The application in human medicine is more realistic, since a combination of biodegradability, hydrophobicity and biocompatibility, together with other interesting specific properties seems to be relevant [25, 214, 216]. PHB composites with apatite can be used as biodegradable bone fracture fixations or even as bone repair materials [217, 218], taking into account the piezoelectric properties of PHB, which are similar to those of bones. Another potential area is controlled drug release, where the advantages of PHAs are far from being fully exploited [215].

PHB fibres were considered to be mainly used for the production of scaffolds [219]. From the point of view of medical applications, an interesting paper by Schmack *et al*. deals with the effect of electron beam irradiation on the properties and degradation of PHB fibres with the aim of estimating the consequences of sterilization of medical devices by that technique. The application as scaffolds was also suggested for centrifugally spun fibres [207], since the fibres, treated with acid or alkali, had rather good adhesion towards cells and could be potentially used as wound scaffold. In this context, an interesting procedure [220] called upon PHA foam prepared by solution casting and subsequent solvent leaching of water-soluble particles, to be applied as scaffold in tissue regeneration. A very well-controlled open pore structure was purported to be suitable for this particular purpose. Other applications of PHAs have been reviewed [128, 212].

22.10 PRODUCERS

Several companies offer PHAs on a commercial basis, as seen in Table 22.12, although their production involves marginal amounts. Among these, Procter & Gamble is the only company producing higher PHAs, namely poly(hydroxyl-3-butyrate-*co*-hexanoate) under the trade name Nodax. The other companies offer PHB or PHBV. Monsanto produces around 800 tons per year of various grades of Biopol, using *Ralstonia eutropha*, having taken over the process developed by ICI and used for years by Zeneca Bio Products, a company formed by the partial splitting of ICI in 1993 [1]. Little is known about the production of Enmat made in China. Biomer in Munich produces the PHB and PHBV of the same trademark, which are reported to be top quality and of excellent purity, but expensive.

A range of PHBV copolymers with valerate comonomer contents varying between 0 and 24 per cent are also produced and sold in the US [221] and in Japan [222]. PHB was also produced on a commercial scale by Chemie Linz for a few years starting in the late 1980s. Unlike the ICI/Zeneca process, the fast-growing bacteria *Alcaligenes latus* were used, which synthesized the homopolymer at a much higher rate [223]. Somewhat misleading information relates to the Brazilian production. On the one hand, the web site [224] claims that the company is called Copersucar and the product Biocycle. On the other hand, a presentation authored by Braunegg, Bona, Kutschera and Ortega, which can be found on another web site [225] presents Biocycle as part of PHB Industrial S/A. Moreover, the production is quoted as taking place in Serrana in the state of Sao Paolo, whereas PHB Industrial S/A is owned by Irmaos Biagi S/A and the Balbo Group, which are large Brazilian producers of sugar and ethanol, with an equal 50 per cent share. Although the data on the company itself are not quite clear in the source cited, the process is well described, including its economy. *Wautersia eutropha* (formerly *Ralstonia eutropha* or *Alcaligenes eutrophus*) are used as the polymer-producing bacteria. The substrate is sucrose made from sugar cane, providing 0.33 kg of PHB per kg of sucrose. Since the yield of sucrose from sugar cane is estimated at 10.8 tons per hectare, the production of PHB per hectare is 3.6 tons. A power plant is also a part of the sucrose production, with bagasse, a waste of sugar cane, being used as fuel. The overall energy balance shows a

Table 22.12

Companies offering PHAs on a commercial basis. Information taken from www.biodegrad.net/biopolymer

Company	Product	Country	Web site
Monsanto–Metabolix	Biopol	USA	www.monsanto.com
Metabolix/ADM	Metabolix	USA	www.metabolix.com
Tianan	Enmat	China	www.fs-tianan.com
Copersucar	Biocycle	Brazil	www.copersucar.com.br
Biomer	Biomer L	Germany	www.biomer.de
Procter & Gamble	Nodax	USA	www.pg.com

total energy production of 12.61 MWh ha^{-1}, while the consumption is 0.95 for the sugar mill and 3.60 MHh ha^{-1} for the PHB production. Thus, a surplus of 8.06 MWh ha^{-1} is left, which can substantially improve the economy of the process. The pilot plant started in 1995 and in 2000 the scaling up raised the production to 50 t PHB per year in 2000. The construction of an industrial large-scale plant should start in 2007, and its production is planned to begin in 2008 with a starting annual output of 4 000 tons.

Currently, the P(4HB) homopolymer has been commercially produced by Tepha Inc., USA, in a large-scale fermentation facility of genetically engineered *E. coli* and developed as a new absorbable material for implantable medical applications [226].

22.11 OUTLOOK

PHAs, and especially PHB/PHBV, promise to be interesting materials for the future development of the plastic industry. However, in spite of a long history and growing interest, these materials seem to be far from reaching a large-scale production. The problems are associated with, on the one hand, some incompletely solved features regarding their properties, especially the design of appropriate processing technologies in relation to their low thermal stability and, on the other hand, the search for proper thermal stabilization, improving the toughness of PHB or, alternatively, lowering the price of all these materials.

It seems that although a number of medical applications are becoming a reality, their required amount of PHAs is not sufficient for running a plant at an economically feasible capacity, estimated to be at least 20 000 tons per year.

The market is ready for biodegradable plastics in many different areas of application, if they prove to have acceptable processing parameters, ultimate properties and viable prices. Since other biodegradable plastics are developing rapidly, for example polylactic acid (see Chapter 21) and starch (see Chapter 15), if PHAs should aspire to be among them, their competitive industrial production should start soon.

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Proteins as Sources of Materials

Lina Zhang and Ming Zeng

ABSTRACT

Proteins are natural, renewable, and biodegradable polymers which have attracted considerable attention in recent years in terms of advances in genetic engineering, eco-friendly materials, and novel composite materials based on renewable sources. This chapter reviews the protein structures, their physicochemical properties, their modification and their application, with particular emphasis on soy protein, zein, wheat protein, and casein. Firstly, it presents an overview of the structure, classification, hydration–dehydration, solubility, denaturation, and new concepts on proteins. Secondly, it concentrates on the physical and chemical properties of the four important kinds of proteins. Thirdly, the potential applications of proteins, including films and sheets, adhesives, plastics, blends, and composites, etc. are discussed.

Keywords

Protein, Protein structure, Physicochemical properties, Industrial application, Denaturation, Soy protein, Zein, Wheat protein, Casein, Films, Adhesives, Plastics, Blends, Composites, 'Green' materials, Biodegradability

23.1 INTRODUCTION

The term protein comes from the Greek προτειυσ. Proteins, as naturally occurring polymers, are important renewable resources produced by animals, plants, and bacteria. In terms of potential sources of materials, soy protein, zein, and wheat proteins (WP) are among the main plant proteins; casein, collagen protein, and silk fibroin represent relevant animal proteins; and lactate dehydrogenase, chymotrypsin, and fumarase constitute major bacterial proteins. A number of proteins have received much attention for the production of biodegradable polymers but, few have led to actual industrial scale-up due to performance difficulties and high production costs. In this chapter, an attempt is made to review the materials based on proteins derived from renewable resources abundantly available in nature, with particular emphasis on soy protein, zein, WP, and casein [1–4].

23.2 STRUCTURES

23.2.1 Primary structure

Proteins are natural macromolecules consisting of 20 different amino acid residues arranged in a highly sophisticated three-dimensional structure. Protein structures can be described at four levels as shown in Fig. 23.1 [5], including the primary structure (amino acid sequence), the secondary structure (conformation), the tertiary structure

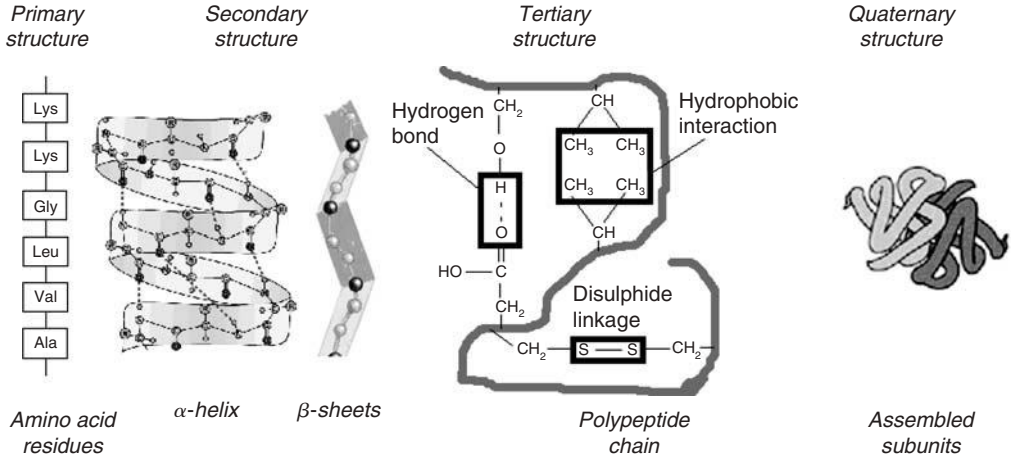

Figure 23.1 Proteins levels. (Reprinted with permission from Reference [5].)

(overall folding of the polypeptide chain), and the quaternary structure (specific association of multiple polypeptide chains). Amino acids have two functional groups, namely, an amino group (—NH_2) and a carboxyl group (—COOH). These groups are joined to a single (aliphatic) carbon, so the amino acids in proteins are all α-amino acids. The sequence of amino acids in a protein is termed its primary structure, and proteins are linear macromolecules formed by linking the α-carboxyl group of one amino acid to the α-amino group of another amino acid with a peptide bond (also called an amide bond). A peptide bond has several properties, such as resistance to hydrolysis, a planar geometry, a hydrogen-bond donor (the NH group) and a hydrogen-bond acceptor (the carbonyl group), the distinctive hydrogen bonding, and the uncharged peptide bond, which allows proteins to form tightly packed globular structures.

23.2.2 Secondary structure

The secondary structure of proteins includes helices, sheets, turns, loops, and random coils. The two most important secondary structures of proteins, the α helix and the β sheet, were predicted by Linus Pauling in the early 1950s. Pauling and Corey recognized that the folding of peptide chains, among other criteria, should preserve the bond angles and planar configuration of the peptide bond, as well as keep atoms from coming together so closely that they repelled each other through van der Waals interactions. Finally, Pauling predicted that hydrogen bonds must be able to stabilize the folding of the peptide backbone [6]. The α helix, a rod like structure, is formed by the hydrogen bonding of the backbone carbonyl oxygen of each residue to the backbone NH of the fourth residue along. The backbone atoms pack closely and form favourable van der Waals interactions, while the side chains project out from the helix. The amino acid residue, proline (without an NH group), interrupts the hydrogen-bonding pattern, leading to a kink in a helix. There are 3.6 residues per turn in the α helix; in other words, the helix repeats itself every 36 residues, with 10 turns of the helix in that interval. The pitch of the α helix is 1.5 Å and the number of residues per turn (5.4) is 3.6.

A β pleated sheet differs markedly from the rod like α helix. A polypeptide chain in a β sheet is almost fully extended rather than being tightly coiled as in the α helix. The β sheet involves hydrogen bonding between backbone residues in adjacent chains. In the β sheet, a single chain forms hydrogen bonds with its neighbouring chains, with the donor (amide) and acceptor (carbonyl) groups pointing sideways rather than along the chain, as in the α helix. The distance between adjacent amino acids along a β strand is approximately 3.5 Å. A β sheet is formed by linking two or more β strands by hydrogen bonds. In the β strand, the polypeptide chain is nearly fully extended.

Most proteins have compact, globular shapes, 'non-regular' structures requiring reversals in the direction of their polypeptide chains [7]. To make a spherical fold for globular proteins, the residues between regular helices and strands need to make sharp turns. Turns, reverse turns, or β-turns, were first recognized by Venkatachalam *et al* [8–10].

23.2.3 Tertiary structure

The tertiary structure of proteins describes the pattern of folding of secondary structures into a compact, more sophisticated molecule that can carry out biological functions. The tertiary structures of water-soluble proteins have the following common morphological features: (1) an interior formed of amino acids with hydrophobic side chains, and (2) a surface formed largely of hydrophilic amino acids that interact with the aqueous environment, directed by the hydrophobic interactions between the interior residues.

23.2.4 Quaternary structure

The tertiary-structured proteins may further associate into a higher degree of complexity, called quaternary structure. In most cases, the subunits are held together by non-covalent bonds. For example, haemoglobin has a molecular weight of 64 000 and is composed of four subunits, each of molecular weight 16 000. Salt bridges, hydrogen bonds, hydrophobic, and van der Waals interaction act in an additive fashion to specifically associate the subunits.

23.3 PHYSICOCHEMICAL PROPERTIES

23.3.1 Classification

Proteins can be assigned to one of three global classes, on the basis of shape and solubility: fibrous, globular, or membrane [11]. Fibrous proteins tend to have relatively simple, regular linear structures, and often serve structural roles in cells. The fibrous protein keratin forms structures such as hair and fingernails. The springy nature of wool is based on its composition of α helices that are coiled around and crosslinked to each other through cystine residues. Globular proteins are roughly spherical in shape. The polypeptide chain is compactly folded so that hydrophobic amino acid side chains are in the interior of the molecule and the hydrophilic side chains are on the outside exposed to the solvent. Consequently, globular proteins are very soluble in aqueous media. Globular proteins, such as most enzymes, usually consist of a combination of the two secondary structures, with important exceptions. For example, haemoglobin is almost entirely α-helical, and antibodies are composed almost entirely of β structures.

Membrane proteins are found in association with the various membrane systems of cells. For interaction with the non-polar phase within membranes, membrane proteins have hydrophobic amino acid side chains oriented outwards, and are insoluble in aqueous media.

23.3.2 Hydration and dehydration

Protein–water interactions play an important role in the maintenance of the three-dimensional structure of proteins, and their study has provided significant advances in our understanding of the involvement of water in protein functionality, stability, and dynamics [12]. Protein hydration is a process from its dry state to the solution state, which occurs over a very wide water activity range. For example, serine can be both a hydrogen-bond acceptor and donor, and is soluble in water as a result of the formation of hydrogen bonds. Serine on the inside of a protein, away from water, can form hydrogen bonds with other amino acids. Water molecules bind to both polar and non-polar groups in proteins via dipole–dipole, dipole-induced dipole, and charge–dipole interactions. The hydration of a protein, therefore, is related to its amino acid composition and is affected by solution conditions such as pH, temperature, and ionic strength [13]. Lyophilized proteins cannot go beyond their lowest hydration level of about 0.01 g H_2O per gram protein, namely about 8 mol of water per mole of protein [14]. When the protein is dehydrated to a certain level, its conformational flexibility decreases in order to maintain a local free-energy minimum. Therefore, the level of hydration significantly affects the biological properties (*e.g.* enzyme activity) of proteins [15]. In general, enzymes require only a small amount of water to express their catalytic activity [16].

The functional properties are illustrated by the water–protein interactions of soy protein isolate (SPI). SPI, with high solubility, or excessive thermally induced insolubilization, or compact calcium-induced aggregates, gives rise to low water-imbibing capacity (WIC) values. The highest WIC results from the balance between intermediate

solubility and the formation of aggregates with good hydration properties. The hydration properties and viscosity of the SPI suspensions are strongly determined by the amount and properties of the insoluble fraction [17, 18].

23.3.3 Solubility

The solubility of proteins is an important property that affects and predicts other functional properties. The solubility of proteins is a thermodynamic manifestation of the equilibrium between protein–solvent and protein–protein interactions. Hydrophobic interactions promote protein intermolecular interactions, resulting in a decreased solubility. Polar and charged amino acid residues, on the other hand, promote protein–water interactions, resulting in increased solubility. As a rule, proteins containing more polar and charged groups, globular in shape, with relatively small molecular weights, have better solubility. The highly hydrophobic proteins, proteins with random structure, or highly aggregated protein polymers, are generally insoluble or unstable in solution. Many thermodynamic variables, such as temperature, pH, ionic strength, and other parameters of the solution system, affect protein solubility and compatibility. At constant pH and ionic strength, the solubility of most proteins increases with an increase in temperature. Intermolecular interactions significantly affect the overall solubility of the system, especially when the protein concentration is high. Processing equipment and stirring conditions also contribute to protein hydration and solubilization. The added energy for stirring gives a high degree of deformation of dispersed particles with a low (related to the dispersion medium) viscosity in flow. In the shear field of a protein solution system, the macromolecules may orient themselves, interact with each other more intensively, and self-associate or dissociate frequently. A weak association of protein molecules may break down in a shear field, thereby increasing the cosolubility of proteins in a multicomponent solution system.

23.3.4 Denaturation

Native proteins having biological functions can exist only in living organisms. With respect to the overall structure of a native protein, any conformational change could mean a certain degree of denaturation. The loss of structural order in these complex macromolecules, so-called denaturation, is accompanied by a loss of function. The nitrogen solubility index, or protein dispersibility index, expresses the percentages of the total content of nitrogen and protein, respectively, and also indicates the extent of protein denaturation. A more 'restrictive' definition of denaturation, therefore, specifies the loss of the most characteristic properties of the protein, such as enzyme activity and solubility. Theoretically, proteins have to maintain their natural conformation to perform their specific functions, hence any factor that changes the conformation of proteins is a potential source of denaturation. Therefore, the denaturation of proteins includes thermal denaturation, denaturation by changing the pH, by using high concentrations of urea, guanidinium chloride and other guanidinium salts, inorganic salts, by organic solvents, and by detergents. Heat is the most common factor that causes the denaturation of proteins. Heat treatment of proteins increases molecular motion, leading to the rupture of various intermolecular and intramolecular bonds of the native protein structure. As a physicochemical process, protein denaturation can be reversible or irreversible, depending on the process of denaturation and the conditions after denaturation. Denatured proteins may refold back to their original structure and resume their biological functions. The process of protein folding, unfolding, and refolding is still an attractive research area, although it has been extensively studied for decades [19]. Methods normally used to denature proteins include exposure to heat, acid/alkali treatment, or the addition of organic solvents, detergents and urea. Wet and dry heat, grinding, freezing, pressure, irradiation and high frequency sound waves can also be used for denaturing proteins [20].

23.4 IMPORTANT PROTEINS AS SOURCES OF MATERIALS

23.4.1 Soy protein

The soybean plant called 'The Gold that Grows', originated in China, has attracted attention in recent years because of its versatile uses [21]. Soybeans contain approximately 42 per cent protein, 20 per cent oil, 33 per cent carbohydrates, and 5 per cent ash on a dry basis. The storage soy proteins consist of a mixture of proteins (α-,β-, and

γ-conglycinins, glycinin, and other globulins) ranging in molecular weight from about 140 to 300 kDa and differing in physicochemical and other properties.

Soy protein has an isoelectric point at pH 4.5. The numerical coefficient is the characteristic sedimentation constant in water at 20°C. Four such fractions are separable and are designated as 2S, 7S, 11S, and 15S. The two major fractions are 7S (35 per cent) and 11S (52 per cent). The 7S fraction is highly heterogeneous, and its principal component is β-conglycinin with a molecular mass of the order of 150–190 kDa. The 11S fraction consists of glycinin with a molecular mass of 320–360 kDa. 11S is a quaternary structure composed of three acidic and three basic subunits of 35 and 20 kDa with isoelectric points between pH 4.7–5.4 and 8.0–8.5, respectively. The other minor fractions are 2S (8 per cent) and 15S (5 per cent). The glass transition temperature of soy 7S and 11S globulin fractions, isolated from defatted soy flour (DSF) by the method of Thanh and Shibasaki, was studied as a function of moisture content, using differential scanning calorimetry (DSC). The DSC scans of 7S and 11S fractions with 10 per cent water content showed an endothermic transition at 120°C and 150°C, respectively [22]. There are two glass transitions (T_{g1} and T_{g2}) in the soy protein plasticized with glycerol, corresponding to glycerol-rich and protein-rich domains, respectively. The T_{g1} of the sheets decreases from −28.5°C to −65.2°C with an increase of glycerol content from 25 to 50 wt per cent, whereas the T_{g2} is almost invariable at about 44°C [23]. The radius of gyration of protein-rich domain ranges from 59 to 60 nm, suggesting the existence of a stable protein domain. Their endothermic peaks at about 100°C on the DSC curves are assigned to the evaporation of residual moisture in the samples. The improvement of the functional and the mechanical properties of proteins by altering their molecular structure or conformation through physical, chemical, or enzymatic agents at the secondary, tertiary, and quaternary levels, has been well documented in the literature [24, 25]. Although soybeans have been consumed as food for thousands of years, soy polymer technology can create an age of green plastic in the twenty-first century [26].

23.4.2 Zein

Zein is extracted from corn grain and has been examined as a possible raw material for polymer applications. It is one of the few cereal proteins extracted in a relatively pure form and constitutes a unique and complex material, zein comes from the alcohol soluble protein of corn, classified as a prolamin, and is the principle storage protein of corn and constituting 44–79 per cent of the endosperm protein. Biologically, zein is a mixture of proteins varying in molecular size and solubility, which can be separated by differential solubility to give four related zeins with distinct types:α, β, and γ and δ. Commercial zein is made up of α-zein, which is by far the most abundant, accounting for around 70 per cent of the total. Zein is known for its solubility in binary solvents containing a lower aliphatic alcohol and water, such as aqueous ethanol or isopropanol. The molecular weight of zein lies in the range of 9.6–44 kDa [27]. For zein to reach its full potential, research must find ways to overcome two main problems, *viz*. a prohibitive cost and a poor resistance to water. The latter drawback makes zein unacceptable for some applications for which it had shown great promise in the past, such as the processing of films and coatings. Moreover, zein's soft, ductile nature after being precipitated from a solvent, promises interesting applications as a plastic material, either alone or in blends [28].

23.4.3 Wheat gluten

Wheat gluten is composed of a mixture of complex protein molecules that can be separated into glutenins and gliadins on the basis of their extractability in aqueous ethanol. Gliadin proteins ($M_W < 50$ kDa) provide the viscous component of gluten and constitute a heterogeneous group of proteins characterized by single polypeptide chains associated by hydrogen bonding and hydrophobic interactions, having intramolecular disulphide bonds, and being soluble in a 70 per cent ethanol/water solution. Glutenins comprise a diverse number of protein molecules grouped into high molecular weight subunits from 80 to several million kDa. Hydrogen bonding between the repeat regions of high molecular weight proteins has been found to be responsible for the elasticity of gluten. Glutenins with low molecular weights between 30 and 80 kDa are partially soluble in a 70 per cent ethanol/water mixture. Gliadins and glutenins are present in almost equal quantities in wheat gluten and have similar amino acid compositions, being high in both glutamine and proline. They also have a considerable number of non-polar amino acids containing aliphatic or aromatic groups. These groups, together with a few readily ionizable amino acids, are responsible for the insolubility of gluten in water. The amount, size distribution, and molecular architecture of glutenins and gliadins greatly

influence the rheological, processing, mechanical, and physicochemical properties of gluten [29]. WP are one of the most important resources with the lowest price among plant proteins. They have good viscoelastic properties, strong tensile strength (TS) and excellent barrier properties for gas and water. The investigation of wheat protein based materials has greatly attracted the attention of chemists and material scientists in recent years [30].

23.4.4 Casein

Casein is the major component (80 per cent) of milk, with molecular weights between 1 and 20 kDa and includes four distinct types: α–s1, α–s2, β, and k. Casein is the predominant phosphoprotein that precipitates at pH 4.6 (20°C) and is characterized by an open, random coil structure. By treating acid-precipitated caseins with alkali solution caseinates are produced. Both caseins and caseinates form transparent films from aqueous solutions without any treatment because of their random coil nature and numerous hydrogen bonds. Caseins have shown to be useful in adhesives, micro encapsulation, food ingredients, and pharmaceuticals [31].

23.5 POTENTIAL APPLICATIONS AS MATERIALS

23.5.1 Films and sheets

Soy protein, zein, gluten, rapeseed protein, casein, and collagen are currently being investigated to prepare edible and non-edible films. Most protein films are produced by casting from solutions, in which proteins, plasticizers, and other agents are dissolved in an appropriate solvent. Extrusion, which is widely used in the plastics industry, is an alternative method that needs to be investigated for the industrial production of protein films. This is a challenge to researchers, since few reports have been published up to now. The thermoplastic behaviour of proteins has been exploited to make films by thermal or thermomechanical processes under low moisture conditions. For soy protein films, heat-curing reduces moisture content, water vapour permeability, elongation, and total soluble matter (TSM), and increases total colour difference and TS pressure, individually and interactively with temperature, significantly affects the film moisture content, TS and TSM [32].

The term edible film is defined as a free standing thin layer of edible material which can be used as a food product or a wrapper for foods. As an edible food wrapper, it has many advantages over the conventional non-edible wrappers, including (1) its biodegradable which makes it consumable with the packaged product, (2) its protective shell which preserves the quality of the packaged food and prolongs its shelf life, and (3) the possibility to load it with additives to enhance the sensory and nutritional properties of the food. Edible films from plant proteins have been investigated extensively in recent years and new products are continuously being developed [33].

A notable feature of soy proteins is the strong pH dependence of the molecular conformation and the associated functional properties, such as surface activity, film structure, surface dilatational viscoelasticity and, especially, the rate of adsorption at a fluid interface. Optimum functionality occurs at $pH < 5$, which limits the application of soy globulins as food ingredients [34]. Whole soy flour and apple pectin have also been used as raw materials for producing hydrocolloid edible films. The best ratio between the two components (2:1 mg cm^{-2}, pectin:soy flour) was determined in order to obtain films which could be perfectly handled for their consistence. Films have also been prepared in the presence of transglutaminase, an enzyme able to produce isopeptide bonds among the soy polypeptide chains. The latter films showed a smoother surface and a higher homogeneity, as demonstrated by microstructural analyses, whereas studies of their mechanical properties indicated that transglutaminase increased their strength and reduced their flexibility [35]. Calcium salts crosslinking interactions with SPI and glucono-δ-lactone (GDL) gave rise to edible films with rigid three-dimensional structure. GDL contributed to the formation of a homogeneous film structure due to increased protein–solvent attraction. The TS of calcium-sulphate-treated SPI films (8.6 MPa) is higher than that of calcium-chloride counterparts (6.4 MPa) and then that of the control (5.5 MPa). The puncture strength (PS) of calcium-sulphate-treated SPI films (9.8 MPa) is higher than that of the calcium-chloride counterparts (8.5 MPa) and then that of the control (5.9 MPa). Moreover, SPI films formulated with GDL have a higher elongation at break (39.4 per cent) than that of the control (18.2 per cent). Calcium salts and GDL-treated SPI films have a lower water vapour permeability than that of the control [36].

The addition of a plasticizer to make a very good film is essential, since it modifies the three-dimensional organization of the proteins, decreases their attractive intermolecular forces, resulting in a decreased cohesion, elasticity,

mechanical properties, and rigidity [37–43]. The most used plasticizers are glycerol and sorbitol, and acetamide, used to plasticize soy protein, has also been recently reported [44]. The effects of plasticizers (glycerol, sorbitol, and 1:1 mixture of glycerol and sorbitol) on the moisture sorption characteristics of hydrophilic SPI films have been investigated at three levels of plasticizer concentration (0.3, 0.5, and 0.7 g plasticizer per gram SPI). Under given relative humidity (RH) conditions, films with higher glycerol ratios absorbed more moisture with higher initial adsorption rates, and films with higher plasticizer contents exhibited higher equilibrium moisture contents. Plasticizer and absorbed water loosened the film synergistically, resulting in a higher elongation but a lower TS. Films with lower glycerol contents were more sensitive to RH variations, as compared to those richer in glycerol [45].

The addition of the anionic surfactant sodium dodecyl sulphate (SDS) into glycerin-plasticized SPI leads to changes in TS, solubility, and water vapour barrier properties of the corresponding films. This can be attributed to the disruption of hydrophobic associations among neighbouring protein molecules, as the non-polar portions of the SDS molecules attach themselves onto the hydrophobic amino acid residues within the film structure. It has been demonstrated that adding SDS to film-forming solutions prior to casting, greatly modifies the properties of the ensuing SPI films. In particular, SDS can improve the water vapour barrier ability and the extendibility of the films, both desirable attributes when assessing the potential of such films for packaging applications [46].

Heat treatment is well-known to generate crosslinks in some proteins, such as soy and whey. Indeed, heat favours soy protein crosslinking by disturbing the protein structure and exposing sulphydryl and hydrophobic groups. Sulphydryl groups have been reported to be responsible for the formation of disulphide linkages which generate a three-dimensional network [47]. To elucidate the effect of γ-irradiation on the physicochemical properties, the molecular and mechanical properties of the SPI films were examined after the γ-irradiation of the film-forming solution at various radiation doses. The γ-irradiation causes the disruption of the ordered structure of the soy protein molecules, as well as degradation, crosslinking, and aggregation of SPI in solution, leading to a decrease in its viscosity. However, the mean TS of the SPI films increased by a factor of 2 after γ-irradiation, as a result of the reduction in water vapour permeability by 13 per cent. The microstructures observed by scanning electron microscopy (SEM) showed that the irradiated SPI films had a smoother and glossier surface than the control film [48]. In addition, γ-irradiation combined with thermal treatments have been used to crosslink sterilized biofilms based on SPI (S system) and a 1:1 mixture of SPI and whey protein isolate, WPI (SW system). This double treatment improved significantly the mechanical properties, namely, the puncture strength and puncture deformation, for all types of protein films. The incorporation of carboxymethylcellulose (CMC) also showed a significant improvement in water vapour permeability for irradiated films of the S system and for non-irradiated films of the SW system.

Zein has been tested as a possible polymeric material because of its film-forming ability. Zein-based plastic sheets and films have been formed by extrusion through a slit-die or blowing head. Zein was plasticized with oleic acid and formed into a wet mouldable mass (resin) to feed the extruders. Both single- and twin-screw extruded sheets showed higher elongation at break, lower TS, and lower Young's modulus than non-extruded samples. Stress–strain plots for extruded samples gave evidence of plastic behaviour. Blown film extrusion can be affected by feed moisture content and barrel temperatures. The optimal moisture content was determined at 14–15 per cent, while temperature at the three extruder zones was maintained at 20–25°C, 20–25°C, and 35°C, respectively. Temperature at the blowing head was 45°C. Film samples blown after either single- or twin-screw extrusion indicated similar tensile properties to those of slit-die extruded samples [49].

23.5.2 Adhesives

Protein-based adhesives can be traced back to the beginning of the last century. Blends of adhesive grade soy flour with casein (ground or screened) have been prepared to obtain glues with composite performance for making panels and flush door assemblies. Composite adhesives for making plant fibre boxes for food were obtained by blending SPI with varying amounts of poly(vinyl alcohol) or poly(vinyl acetate) [50]. The formation of electrostatic/covalent complexes upon mixing SPI with sodium alginate/PGA (propylene glycol alginate) under alkaline conditions has been reported [51]. Films formed from covalent complexes had greater stability in water as compared to those obtained from protein–alginate complexes. The adhesive performance of soybean proteins is dependent on the particle size, the nature of surface, the structure of the protein, its viscosity and pH. Other factors, which can affect their performance, are the processing parameters such as the press temperature, the pressure, and the time [52]. The major advantage of soy glues is that they can be cured either hot or cold. The major disadvantages of soybean protein-based adhesives are low gluing strength and poor water resistance [53]. To improve the water performance,

SPIs have been modified using SDS and guanidine hydrochloride (GuHCl). The SDS-modified SPI containing 91 per cent protein has a water-soluble mass of 1.7 per cent. To be considered as a water-resistant adhesive, its water-soluble mass of adhesive should be less than 2 per cent. The wet shear strength test showed 100 per cent cohesive failure within the fibreboard, indicating that the modified SPI had good water resistance. Drying treatment significantly affected the final adhesion performance. Its shear strength did not change much, but the percentage of cohesive failure within the fibreboard increased markedly as the drying temperature was increased. All the unsoaked, soaked, and wet specimens glued by the adhesives treated at 70°C or 90°C had 100 per cent cohesive failure within fibreboard. The viscosity also increased greatly with an increase in the drying temperature [54, 55].

Citric acid (CA) was thermochemically reacted with food quality SPI, distillers dried grains (DDG), produced from corn dry milling, and corn gluten meal (CGM), produced from corn wet milling, to generate acid-stable products with enhanced metal-binding properties. CA dehydrates at high temperature to form the corresponding anhydride that can interact with the nucleophilic functional groups of proteins or carbohydrates to generate ester or acyl derivatives. The effects of temperature, CA concentration, pH, and reaction time were evaluated to show that SPI, DDG, and CGM, when heated in the range of 110–120°C with CA at 1:1 w/w ratio, under endogenous acidic conditions for 24 h, yielded products with reaction efficiencies >60 per cent, which possessed 4.13, 4.19, and 4.26 mmol COOH per gram, respectively. FTIR data of the original heated proteins, compared with their respective CA products demonstrated additional peaks indicative of ester and carboxyl linkages. The SPI/CA, DDG/CA, and CGM/CA products effectively bound 1.18, 1.07, and 0.98 mmol of Cu^{2+} per gram, respectively, when analysed by ion plasma spectrometry. Solid-state NMR supported the metal-binding characteristics of the CA reaction products and demonstrated that Al^{3+} was bound ionically to their carboxyl groups. Amino acid composition studies showed diminished amounts of amino acids with nucleophilic reactive groups in all three CA reaction products. The CA reaction products were highly resistant to acid hydrolysis with 6 N HCl for 4 h at 145°C. Moreover, the products generated possessed cation-exchange capabilities and a potential biodegradability that may have an outlet for industrial wastewater treatment [56].

Recently, urea and urease inhibitor *N*-(*n*-butyl) thiophosphoric triamide (*n* BTPT) were used to modify wheat straw–soy flour particleboards. Boric and citric acid, along sodium hypophosphite monohydrate, were used to modify soy carbohydrates. Particleboard bonded by urea and high concentrations of *n* BTPT-treated soy flour showed improved mechanical properties, whereas that bonded by boric acid-treated soy flour had better water resistance. The adhesive made from soy flour treated with 1.5 M urea, 0.4 per cent *n* BTPT, 7 per cent CA, 4 per cent NaH_2PO_2, 3 per cent boric acid, and 1.85 per cent NaOH, produced particleboard with the maximum mechanical strength and water resistance [57].

23.5.3 Plastics

A great deal of research of soybean plastics was conducted in the 1930s and 1940s. At that time, soybean products were incorporated into phenolic resins mainly as filler or extender to decrease the cost of the plastics, because petroleum was expensive whereas soybeans were abundantly available. Decreasing petroleum prices and better-performing petroleum-based plastics, dominated the market after World War II. At that time, Henry Ford mixed soy protein with phenol–formaldehyde resin to produce automobile body parts [58]. Brother and Mckinney reported making plastics by using soy protein and various crosslinking agents [59]. Most plastics, at present, are petroleum-based and do not degrade over many decades under normal environmental conditions. As a result, efforts towards developing environment-friendly and biodegradable 'green' plastics for various commercial applications have gained significant momentum in recent years. SPI-based 'green' plastics have been shown to suffer from high moisture sensitivity and low strength. These properties have limited their use in most commercial applications.

In addition, SPI-based plastics are also difficult to process into sheets without any plasticizer. Thermoplastic sheets prepared from SPI with ethylene glycol (EG), as the plasticizer, have been obtained by compression moulding under a pressure of 15 MPa at 150°C. With increasing EG content, the TS and Young's modulus decreased and the elongation at break increases. The water resistance of the thermoplastic sheet of SPI increased with an increase in the EG content and was much higher than that of thermoplastic starch sheets or cellulose films. Further investigation has been carried on SPI sheets containing 50 per cent EG, which display a maximum water resistance in boiling water, good mechanical properties, and a light transmittance of 82 per cent at 800 nm because of the interchain hydrogen bonds and novel crystals [60].

The effects of water, glycerol, methyl glucoside, $ZnSO_4$, epichlorohydrin, and glutaric dialdehyde on the mechanical properties of soy protein plastic sheets have been studied. The thermal transition temperatures and dynamic

mechanical properties of soy protein plastics have also been investigated in terms of the effect of the moisture and glycerol contents on their properties. The glass transition temperatures of the sheets varied from *ca.* −7°C to 50°C with moisture contents ranging from 26 to 2.8 per cent and 30 parts. And the moisture range cited is right of glycerol. The soy protein plastic sheets are usually in their glassy states at room temperature, unless they contain a high level of moisture. The β-transitions of soy protein plastic sheets lie in the range of −33°C to −72°C depending on their moisture content. In the presence of two parts of $ZnSO_4$, the water absorption of the soy protein sheets decreased by 30 per cent. Therefore, soy protein sheets absorbed or lost moisture, depending on the RH of the environment [61].

The effect of storage time on the thermal and mechanical properties of SPI plastics was also investigated and showed that the glass transition temperature and the dynamic storage modulus increased and the loss tangent decreased during storage. The excess enthalpy of relaxation of SPI plastics has an exponential relationship with the storage time, indicating a fast aging rate at the beginning of the storage. The SPI plastics containing glycerol have the slowest aging rate and are fairly stable after 60 days, with about 8.8 MPa TS and 168 per cent strain at break. Urea-modified SPI plastics also displayed slow aging and became relatively stable after 60 days, with about 10 MPa TS and 72 per cent elongation [62, 63].

Processing and modification routes of proteins have been used to produce and to improve properties of biodegradable plastics. SPI, acid-treated and crosslinked by acetic acid and glyoxal have been subsequently compounded, extruded, and injection moulded. Heat treatment is also used as a possible methodology to crosslink the protein structure. The moulded specimens are tested in terms of their tensile properties and solubility at different pH, and are also evaluated for their degree of crosslinking and molecular weight distribution. The ensuing plastics are rigid and brittle with stiffness ranging from 1 436 MPa for SPI, to 1 229 MPa for glyoxal crosslinked SPI, up to 2 698 MPa for heat-treated SPI. The solubility profiles have been studied as a function of the pH of the immersion solutions and the crosslinking degree of each material. A reduction in protein solubility with decreasing pH was observed, with a minimum between pH 4 and 5 and a resolubilization of the protein at pH lower than 4 and greater than 8. Higher levels of crosslinking resulted in a decrease in the solubility and an aggregation of the protein molecules [64, 65].

A great deal of research has been recently focusing on the modification of waterborne polyurethane (WPU) with natural polymers including proteins. WPUs grafted, mixed and crosslinked with casein were prepared, respectively, by incorporating casein into the WPU and its prepolymer aqueous dispersion [66–68]. The particle size and content of casein play an important role in enhancing the mechanical properties of the composite sheets. The WPU/casein sheets possessed good mechanical properties, optical transmittance, and miscibility between the two components. Moreover, blend sheets crosslinked with ethanedial were successfully prepared. By introducing ethanedial into WPU/casein (1:1 by weight), the mechanical properties and water resistivity of the blend materials were enhanced, obviously as a result of the formation of a network. When the ethanedial content was about 2 wt%, the blend sheets showed significantly higher TS, water resistivity, thermal stability, and kept roughly the same elongation at break as those of uncrosslinked blends.

Soy dreg (SD) is an abundant by-product from the isolation process of soy protein with only a tenth of the price of SPI in China. Thus, using SD as the raw material for preparing biodegradable plastics is not only helpful in solving the environmental problems, but also in enhancing the value of agricultural by-products. Water-resistant composite plastics have been prepared from SPI or SD, poly(3-caprolactone) and toluene-2,4-diisocyanate as the compatibilizer by blending and one-step reactive extrusion, followed by compression moulding [69]. The resultant SPI and SD composite materials exhibited high water resistance and good TS (14.8 MPa for SPI-35 and 16.3 MPa for SD-35). Moreover, the SD sheets containing cellulose possessed a higher TS than those of the SPI series, when the SD content was 30–35 per cent, whereas the latter had a better biodegradability and water resistance. By burying the two materials in soil and culturing them in a mineral salt medium containing microorganisms, both of them were almost completely degraded.

23.5.4 Blend and composite materials

The blending of two or more polymers, as a simple and convenient procedure, is very important for preparing biodegradable materials based on renewable resources. The general methods include casting from solution, modification during extrusion, semi-interpenetrating polymer networks (semi-IPN), and nanocomposites [70, 71]. Microporous membranes have been prepared by blending cellulose and SPI in an NaOH/thiourea aqueous solution. Cellulose, immersed in a 6 wt% NaOH/5 wt% thiourea was medium, was kept below −8°C for 12 h, and then stirred vigorously at 20°C for 1 h to obtain a cellulose solution. SPI was dispersed in the solvent to form a slurry with an SPI content

of 40–50 wt%. The mixture of the cellulose and SPI solutions was cast on a glass plate to give a gel sheet, and then coagulated with a 5 wt% H_2SO_4 aqueous solution to generate transparent membranes. The blend membranes were hydrolyzed with a 5 wt% NaOH aqueous solution, and dried in air, coded as CS2-40 and CS2-50. The resulting microporous membranes kept a high TS in both dry and wet states. Interestingly, the cellulose/SPI microporous membranes containing a small amount of SPI can be suitable for the culture of Vero cells as shown in Fig. 23.2 [72]. Therefore, the membranes are good candidates for application in separation technology and biomedical fields.

Blends of soy protein and biodegradable polyesters have been prepared using glycerol as a compatibilizing agent. Good miscibility was obtained only when the soy protein was initially combined with glycerol under high shear at elevated temperatures in an extruder. The extrusion conditions and appropriate screw configuration were the critical factors that affected the reactivity of the protein and hence, the properties of the blends. Under these conditions, partial denaturing of the soy protein leads to specific interactions between the functional groups of the protein and the glycerol. Screws with large kneading blocks that produced high shear mixing were preferred and led to thermoplastic blends with high elongation and TS. Moreover, under appropriate processing conditions, even this low grade protein could be compounded to yield low cost, biodegradable materials that could replace low density polyethylene products [73].

The aqueous dispersion of DSF containing soy protein, soy carbohydrate, and soy whey, has been blended with a styrene–butadiene latex to form elastomer composites. The inclusion of soy carbohydrate increased the tensile stress in the small strain region, but reduced the elongation at break. The inclusion of soy carbohydrate and soy whey also improved the recovery behaviour in the non-linear region. At small strain, the shear elastic modulus of 30 per cent filled composites at 140°C, is about 500 times higher than that of the unfilled elastomer, indicating a significant reinforcement effect generated by DSF. Compared with SPI, the stress softening effect and recovery behaviour under dynamic strain indicated that the addition of soy carbohydrate and soy whey had enhanced the filler–rubber interactions [74]. Blends of SPI with 10, 20, 30, 40, and 50 per cent poly(ethylene-*co*-ethyl acrylate-*co*-maleic anhydride) (PEEAMA), with or without the addition of 2.0 wt% methylene diphenyl diisocyanate (MDI), were prepared

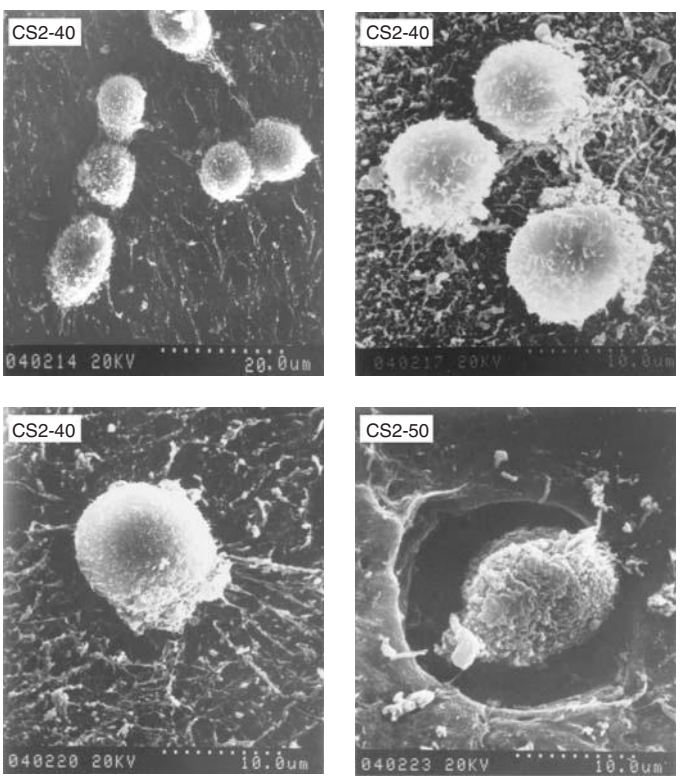

Figure 23.2 SEM photographs of the Vero cells cultured on the free surfaces of the protein based CS2-40 and CS2-50 (see text) membranes. (Reprinted with permission from Reference [72].)

in an intensive mixer at 150°C for 5 min. The blends were then compression-moulded into a tensile bar at 140°C. These materials showed two composition-dependent glass transition temperatures. Furthermore, as the SPI content increased, the melting temperature of the PEEAMA remained constant, but its heat of fusion decreased. These results indicated that SPI and PEEAMA are partially miscible. Increasing the PEEAMA content resulted in a decrease in the modulus and TS and an increase in the elongation and toughness of the blends. Water absorption of the blends also decreased with increasing PEEAMA content. Incorporating MDI further decreased the water absorption of the blends. The mechanism of water sorption of SPI was relaxation controlled, and that of the blends, diffusion controlled [75].

Composites are processed by a variety of methods including compression. Environment-friendly fibre-reinforced composites have been fabricated using ramie fibres and SPI. Based on the interfacial shear strength results and fibre strength distribution, three different fibre lengths and fibre weight contents (FWC) were used to fabricate short fibre-reinforced SPI composites. The fracture stress of the composites increased with an increase in fibre length and FWC. The addition of glycerol increased the fracture strain and reduced the resin fracture stress and modulus as a result of plasticization. The short fibres acted as flaws leading to a reduction in the tensile properties. On further increasing the fibre length and the FWC, a significant increase in the Young's modulus and fracture stress and a decrease in the fracture strain were observed as the fibres started to control the tensile properties of the composites. The ramie fibres and SPI polymers formed compatible moderate-strength composites. However, there is significant scope to improve the tensile properties of both the SPI polymer and the composites containing natural fibres by optimizing the associated processes [76]. Grass fibres were treated with an alkali solution, leading to a more homogenous dispersion of the biofibre in the matrix as well as an increase in the aspect ratio of the fibre in the composite, resulting in an improvement of the mechanical properties, including tensile and flexural properties, as well as impact strength. Additionally, the alkali solution treatment increased the concentration of hydroxyl groups on the fibre surface, resulting in a better interaction between the fibres and the matrix [77].

Nanocomposites have been prepared using a colloidal suspension of chitin and cellulose whiskers as a filler to reinforce SPI plastics with glycerol as the plasticizer. The strong interactions between fillers and between the filler and the SPI matrix played an important role in reinforcing the composites without interfering with their biodegradability. Furthermore, the incorporating of chitin whisker or cellulose whiskers into the SPI matrix led to an improvement in the mechanical properties and the water resistance of the SPI-based nanocomposites [78, 79]. The SPI/chitin whisker nanocomposites, with whiskers having a length of 500 + 50 and a diameter of 50 + 10 on average, respectively, under 43 per cent RH, exhibited a strong increase in both TS and Young's modulus, from 3.3 MPa for a GSPI sheet without chitin whiskers, to 8.4 MPa, and from 26 MPa for the GSPI sheet to 158 MPa, respectively, with increasing chitin whisker content from 0 to 25 wt%, as shown in Fig. 23.3. Further, incorporating of whiskers led to an improvement in water resistance.

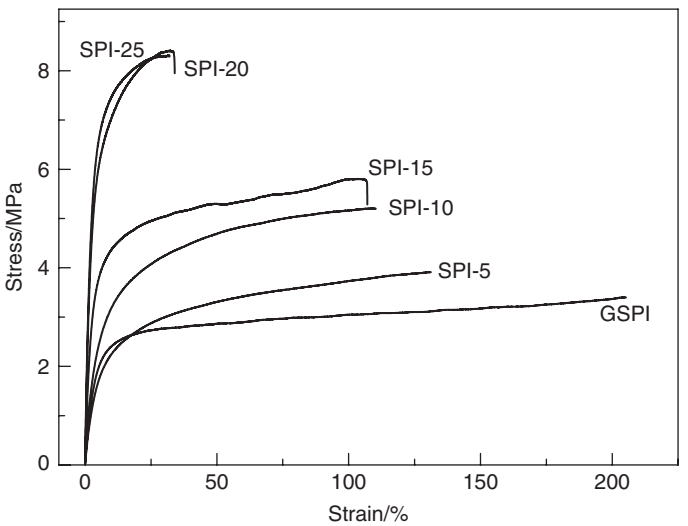

Figure 23.3 Stress–strain curves of GSPI sheet and SPI/chitin whisker nanocomposites conditioned at 43 per cent RH. (Reprinted with permission from Reference [78].

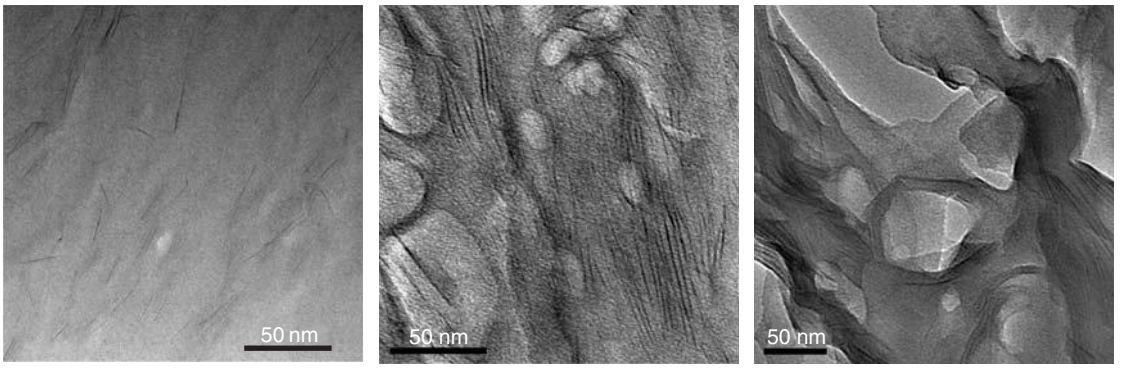

Figure 23.4 TEM images of SPI/MMT plastics, (a) MS-8, (b) MS-16, and (c) MS-24 (see text for the meaning of the symbols). (Reprinted with permission from Reference [80].)

High performance natural polymer/clay materials with an intercalated or highly exfoliated structure have been prepared by several research groups. The heterogeneous distribution of the surface positive charges of SPI provided the possibility for the negatively charged soy protein to intercalate and exfoliate the layered Na^+-montmorillonite (MMT). At least two types of interactions were involved in these composites, namely, surface electrostatic interactions and hydrogen bondings. These highly exfoliated or intercalated SPI/MMT materials were successfully prepared from the solution-intercalated SPI/MMT nanocomposties via extrusion mixing and compression moulding [80]. Using 30 wt% glycerol as a plasticizer, the SPI/MMT plastic sheets were prepared by a compression-moulding process at 140°C and 20 MPa. The X-ray diffraction (XRD) patterns of the nanocomposite plastics (MS-0 to MS-24) with an MMT content varying from 0 to 24 wt%, indicated that the high degree disordered structure of the materials had been maintained in the compression-moulding process. In addition, the diffraction peak of MS-24 shifted to a lower angle ($2\theta \approx 1.4°$), indicating a higher interlayer spacing of 6.2 nm. The microstructure of these materials was visualized by TEM, and the images are shown in Fig. 23.4. For the MS-8 plastic sheet (Fig. 4(a)), the delaminated silicate lamellas were randomized during the extrusion and compression-moulding processes. The dimension of the silicate layers were diminished to about 30 nm in length and 1 nm in thickness, indicating that the layered MMT had been highly exfoliated by the soy protein molecules. For the MS-16 plastic sheet (Fig. 4(b)), most of the layered MMT tactoids were intercalated with a *d*-spacing of about 6 nm. Simultaneously, some conglomerations of MMT occurred within the soy protein matrix. When the MMT content reached 24 wt%, the degree of conglomeration became serious in the MS-24, leading to the obvious phase separation between the components (Fig. 4(c)). Moreover, their thermal stability and mechanical properties were improved as a result of the fine dispersion of the MMT layers and the strong restriction effects on the interfaces. The values of the Young's modulus increased from 180 to 540 MPa by increasing the MMT content from 0 (MS-0) to 16 wt% (MS-16), and the TS increased from 8.8 MPa for the MS-0 to 15.4 MPa for the MS-16 sheet. Within the whole MMT content range, the elongation at break kept decreasing with increasing MMT content. All these results confirm that the strong electrostatic and hydrogen bonding interactions between the soy protein and the highly dispersed MMT layers were sufficient to restrict the segmental motions of the soy protein macromolecules, leading to the improvement of their modulus and TS. The thermal stability of these materials showed a weight loss between 0°C and 120°C, attributed to their absorbed moisture, followed by a second weight loss at 120–250°C, mainly related to the evaporation of glycerol. Thereafter, the pure SPI material is less important than that of the material with MMT.

Mixtures of SPI and native or modified (crosslinked) maize starch have been extruded in a twin-screw extruder at screw speeds of 80, 120, and 160 rpm and a moisture content of 250 g kg^{-1} (dry basis). The specific mechanical energy dissipation and the water solubility index of the ensuing blends were lower than those of native starch/SPI and their sectional expansion indices higher. The type of starch mixed with soybean also affects the expansion of the extrudates, and another factor, in addition to phase transition, could be related to the effect of soybean on that expansion. The effect of the presence of modified starch on the expansion of extrudates can result from the reduction in the specific mechanical energy dissipation, or the higher resistance of modified starch to degradation. No effect of increasing screw speed on the specific mechanical energy dissipation into the feed during extrusion

was observed, as the flow rate of the feed was not held constant [81]. Extruded samples of starch–casein blends were processed by using a single-screw extruder. The independent variables of the process, namely, temperature (126–194°C), moisture content (18–29 per cent), and starch–casein composition (5–95 per cent), affected significantly the physicochemical and textural properties of the ensuing blends. The highest values for expansion and water absorption index were found when a higher starch proportion was present in the blends, at a barrel temperature of 126°C and a moisture content higher than 25 per cent. By increasing the barrel temperature from 126°C to 194°C, the water solubility index and colour parameter were increased. The compression force (CF) was found to be strongly dependent on moisture content and casein proportion in the blend, the highest CF values being found at starch concentration around 50 per cent and 25 per cent moisture content [82].

SPI–lignosulphonate (LS), alkaline lignin and SPI–LS–celloluse blends exhibited higher TS, elongation, and Young's modulus than the corresponding material containing only SPI. The improvement of these properties is attributed mainly to the existence of intermolecular interactions among the components, to a beneficial microphase separation, and to the formation of a physically crosslinked network between SPI and LS. Therefore, a physical network between SPI molecules and an active LS molecule placed at its centre is proposed as the most plausible structure [83].

Thermally processed WP and polyvinylalcohol (PVOH) blends have been studied by solid-state high resolution NMR spectroscopy. The intermolecular hydrogen bonding interactions between WP and PVOH induced some miscibility in the system at the nanometre scale, especially when the PVOH content was low. The TS and modulus of the blends were improved when compared to those of WP. However, the intermolecular interactions were relatively weak and could not be further enhanced by increasing the PVOH content, because such an increase enhanced the immiscible character of the blend composites [84].

Water-blown low density rigid polyurethane foams have been prepared with poly(ether polyol)s, polymeric isocyanates, DSF, water, a catalyst mixture, and a surfactant the immiscible character of the blend composites. Soy flour and the initial water content were varied from 0 to 40 per cent and from 4.5 to 5.5 per cent of the poly(ether polyol) content, respectively. The addition of soy flour in the rigid polyurethane foam system contributed to a higher glass transition temperature, and increasing the initial water content also resulted in an increase in the glass transition temperature [85].

SPI, soy fibre, and corn starch together with 0–40 per cent polyether polyol were also incorporated into a flexible polyurethane foam formulation. Stress–strain curves of the control foam and foams containing 10–20 per cent biomass material exhibited a considerable plateau stress region, but not for foams extended with 30–40 per cent of them. An increase in the biomass content produced an increase in the foam density, whereas an increase in the initial water content produced the opposite effect. Foams extended with 30 per cent SPI, as well as those extended with 30 per cent soy fibre, displayed considerably higher resilience values than all other extended foams. The comfort factor increased by increasing the biomass content, and foams containing 10–40 per cent biomass showed significantly lower values in compression-set than the control foam [86].

A novel series of protein composites have been prepared from 30 to 50 wt% polyurethane prepolymer (PUP) with SD, soy whole flour (SWF) and SPI, by a compression-moulding process at 120°C, without the addition of any plasticizer. The toughness, thermal stability, and water resistivity of these composites was significantly improved. By increasing the PUP content, elastomeric materials could be obtained. With an increase in cellulose content in the system, the TS and water resistivity of the ensuing composites increased [87].

It is particularly interesting to focus on the organization at the interface zein matrix/starch granule, in order to understand the physicochemical properties involved in the formation of cereal endosperm. To study the influence of the starch–zein ratio on material properties, corn flour and starch–zein (5–50 weight per cent) based samples were prepared by extrusion and thermomoulding and then analysed at a moisture content of 12.0 per cent (wet basis). Amorphous starch was ductile, whereas blends and corn flour samples were brittle. The blend morphology, observed by confocal scanning light microscopy, showed that the proteins had to undergo aggregation during the thermomechanical processing, which largely conditioned the mechanical properties of the ensuing materials [88].

Casein has been found to be an excellent candidate to produce oil-in-water emulsions that have both high physical and oxidative stability. The differences in the physical properties and oxidative stability of corn oil-in-water emulsions stabilized by casein, WPI, or SPI at pH 3.0, have been investigated. Emulsions have been prepared with 5 per cent corn oil and 0.2–1.5 per cent protein. Physically stable, monomodal emulsions have been prepared with 1.5 per cent casein, 1.0 or 1.5 per cent SPI, and $\geq$0.5 per cent WPI. The oxidation stability of the different protein-stabilized emulsions was in the order of casein $>$ WPI $>$ SPI, as determined by monitoring both lipid hydroperoxide and headspace hexanal formation. The degree of positive charge on the protein-stabilized emulsion droplets was not the only factor involved in the inhibition of lipid oxidation, because the charge of the emulsion droplets

(WPI > casein ≥ SPI) did not parallel the corresponding oxidation stability. Other potential reasons for the differences in the oxidation stability of the protein-stabilized emulsions include differences in the interfacial film thickness, protein chelating properties, and differences in the free radical scavenging amino acids [89].

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– 2 4 –

Polyelectrolytes Derived from Natural Polysaccharides

Marguerite Rinaudo

ABSTRACT

This chapter describes the main properties and methods for the characterization of polyelectrolytes derived from the biomass. The most important sources are plants, with cellulose and starch, which turn to polyelectrolytes after chemical modifications. Carboxymethylcellulose is the main cellulose derivative used in many industrial applications as good thickener and hydrophilic polymer for aqueous media. Cationic starches are mainly used in the paper industry for filler retention or paper wet-strength. Natural polyelectrolytes are produced by algae with anionic alginates and carrageenans as the major representatives, which are used in food applications and for biomedical devices. In this respect, alginates are often associated in an electrostatic complex with a pseudo-natural polyelectrolyte (chitosan), a cationic polymer extracted from crustaceous shells.

Keywords

Polyelectrolyte, Carboxymethylcellulose, Pectin, Carrageenan, Alginate, Cationic starch, Galactomannan, Lignosulphonate, Ion exchange, Conformational transition, Physical gelation, Electrostatic complex, Chitosan

24.1 INTRODUCTION

Plant biomass, including algae, is a very important source of renewable polysaccharides, some of which have original properties compared with those of synthetic polymers, as shown in Table 24.1 [1–3]. Cellulose and starch are discussed respectively in Chapters 16 and 15. The corresponding animal-derived chitin [4] is covered in Chapter 25. The microbial water soluble polysaccharides like hyaluronan, xanthan and succinoglycan [1, 2, 5] are dealt with in Chapter 13. This chapter focuses on the polyelectrolytes based on polysaccharides from plant and algae.

24.2 POLYELECTROLYTE CHARACTERIZATION

When a polymer bears ionic groups regularly appended on its chain, it is called a polyelectrolyte. The parameter which controls its thermodynamic properties in solution is the charge parameter λ proportional to the linear charge density, introduced in the polyelectrolyte theory proposed by Katchalsky [6] and later by Manning [7]. It is expressed as:

$$\lambda = (\nu/h)(e^2/D\mathrm{kT}) \tag{24.1}$$

where ν is the number of ionic charges along a chain with a contour length h, e is the electronic charge, D is the dielectric constant taken as that of water ($D = 78$) and kT is the Boltzman term; λ is also written as $\lambda = (\nu/h)Q$

Table 24.1

The most important natural polysaccharide and lignin sources

Sources	Polymer extracted	Initial polymer or derivatives	Main properties
Wood	Lignin	Alkali-lignin, Lignosulphonate (from sulphite paper process)	Clay deflocculant Additive in drilling fluids
	Cellulose	Fibres: textile, paper, composites Derivatives: methylcellulose, carboxymethylcellulose….	Fibrous substrate Gelling and/or thickening polymers
	Hemicelluloses		
Fruits	Pectins	Copolymer based on galacturonic acid repeat units with different degrees of methylation	Gelling polymer depending on the cations and temperature
Algae	Carrageenans (extracted from red algae)	Sulphated alternated copolymers (λ,κ,ι forms)	Thickening or gelling polymers depending on the cations and temperature
	Alginates (extracted from brown algae)	Block copolymers based on guluronic and mannuronic units	Gelling polymer in the presence of divalent counterions
Cereals	Starch	Cationic starch	Fibres and fillers retention in paper industry
	Cellulose	Fibres: textile, paper, composites Derivatives: methylcellulose, carboxymethylcellulose….	Fibrous substrates Gelling and/or thickening polymers

with Q, the Bjerrum length, that is 7.2 Å at 25°C in aqueous solution. Hence, λ is directly imposed by the distance b between two ionic sites projected on the axis of the chain (b is the length of a monomeric unit if each monomer has an ionic charge); in the present context, the ionic sites are mainly —COO^- (in carboxymethylcelluloses, pectins and alginates), —NH_3^+ (for chitosan in acidic media) and —SO_3^- (in carrageenan). In the case of polyelectrolytes with —COO^- and —NH_3^+ functions, the net charge will depend strongly on the pH, in connection with the dissociation equilibrium. The electrostatic potential of the polyelectrolyte grows progressively as the degree of polymerization increases and goes to a limit as soon as the number of charges (or degree of polymerization) is larger than 15. Then, the thermodynamic properties become independent of the molecular weight.

In addition, b is directly related to the conformation of the polymer; viz. single or double chain helix formation implies a decrease of the length b. This helical conformation, which often exists in stereoregular polysaccharides, is stabilized by an intrachain and interchain H-bond network. Thus, the study of electrostatic properties will help to characterize the conformation of these polymers as a function of the experimental conditions (pH, ionic concentration, temperature) [8, 9]. One of the most useful experiments is the determination of the activity coefficient of counterions (γ) obtained by potentiometry (or conductimetry). Its theoretical value is directly related to the charge parameter and the valence of the counterion. The main relations, when λ is higher than unity, are:

$$\gamma_1 \sim (2\lambda)^{-1} \quad \text{for monovalent counterions} \tag{24.2}$$

and

$$\gamma_2 \sim (4\lambda)^{-1} \quad \text{for divalent counterions} \tag{24.3}$$

When this experiment is combined with additional determinations, such as optical rotation, which indicates that an ordered conformation is formed in a given situation and molar mass (one or two chains are associated), the determination of this parameter, inversely proportional to b, gives information on the conformation of the chain, allowing

to conclude whether it is a single helix, a double helix on itself or a double helix (or a helical dimer) made of two chains. A double helix was clearly demonstrated with κ-carrageenan, as discussed later [10].

Differential scanning calorimetry (DSC), circular dichroism and NMR spectroscopy are convenient methods to demonstrate the existence of a helical conformation (or at least the existence of an ordered conformation in a given situation) and to determine the thermodynamic conditions for the helix–coil transition [11, 12].

An original behaviour in salt-free solution is observed by viscometry or light scattering and neutron scattering. A peak is observed in the reduced viscosity plotted as a function of polymer concentration, as shown in Fig. 24.1; these results were obtained with the sodium salt of a short polygalacturonate. First, each curve passes through a maximum, whose position depends on the external salt concentration: it was demonstrated that the maximum is located at $C_p = 2C_s$ or $2\lambda C_s$ (when λ is larger than 1) [13]. C_p is the polymer concentration expressed in charge equivalents per litre. On the right side of the peak, interchain electrostatic interactions are established between the chains in solution whereas, on the left side, chains are progressively diluted and controlled by the external salt concentration which remains constant and high in comparison with the polymer concentration. Under the conditions corresponding to the right side of the viscosity peak, a peak (at q_{max}) is observed in light or neutron scattering (the q vector range necessary to evidence this peak depends on the range of polymer concentrations covered) [14, 15]. q_{max} increases when the polymer concentration increases, indicating that a preferential distance ($d \sim q_{max}^{-1}$) exists in solution in the absence (or at low concentration) of external salt. The position of the peak, q_{max}, increases as a function of $C^{1/2}$ (C being the polymer concentration), as predicted for a hexagonal packing. This peak is suppressed in the presence of an excess of salt, when C_s is larger than the value corresponding to $C_p/C_s \sim 2$ or 2λ (when λ is larger than 1). The position of the viscosity peak was shown to be independent of the molar mass of the polymer [8, 16].

The original behaviour also concerns the ionic selectivity in some polysaccharides, which is related to the formation of ion pairs and which occurs in the range of charge parameters larger than 1. This feature was demonstrated from ultrasound experiments on carrageenans [17] and alginate, and also on carboxymethylcelluloses (CMC) [18]. However, for $\lambda < 1$, selectivity was shown even in the coiled conformation for the K^+ and Na^+ forms of κ-carrageenan and at the same time it was found that K^+ formed more ion pairs and favoured the double helix formation compared with Na^+.

From all these original properties, it follows that to characterize a polyelectrolyte in aqueous solution for its molar mass and/or dimensions, it is necessary to isolate the chains by screening the long-range electrostatic repulsions. This is achieved in the presence of 0.05 M, or better 0.1 M, monovalent external salt (NaCl, $NaNO_3$, etc.), allowing SEC and viscometry to be used as normally with neutral polymers.

Another very important point in this type of study is the purification technique adapted to ionic polysaccharides, which involves the exchange of multivalent counterions with monovalent ones and the prevention of aggregate formation mainly due to the large amount of —OH groups in polysaccharides forming cooperative H-bonds [19].

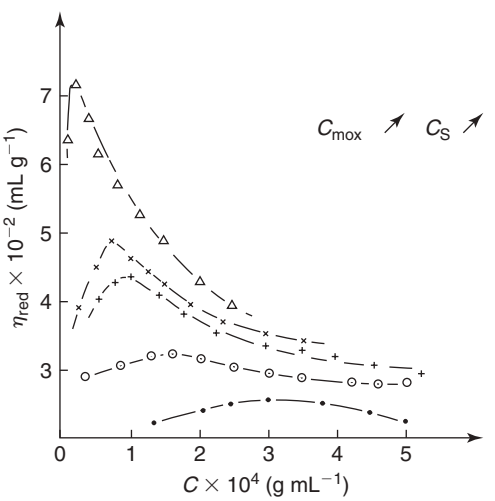

Figure 24.1 Variation of the reduced viscosity for sodium polygalacturonate (Mw = 25 500) as a function of the polymer concentration at different NaCl concentrations. •, 5×10^{-4} M; ○, 2×10^{-4} M; +, 1×10^{-4} M; ×, 5×10^{-5} M; Δ, 1×10^{-5} M. (Reproduced from Reference [13], with the permission of ACS.).

The rheology of many polysaccharides is particularly interesting compared with that of synthetic polymers because of their local stiffness which imposes a large intrinsic viscosity for a given molecular weight. The worm-like chain model was applied to analyze the behaviour of ionic polysaccharides and to characterize their local stiffness by a persistence length L_t, corresponding to the value at the ionic concentration considered: $L_t = L_p + L_e$, where L_p is the intrinsic persistence length obtained when L_t is extrapolated to infinite salt concentration to screen the electrostatic contribution (L_e), assuming that the θ-conditions are approached. Concurrently, the L_p value was calculated for a few polysaccharides by molecular modelling and found to be in good agreement with the experimental values [20–22]. For alginates rich in G units, L_p was found to be 9 nm whereas for a sample rich in M units, L_p decreased to 4 nm [23].

This treatment allows one to predict the dimensions of the chain (and particularly the radius of gyration R_g) and the intrinsic viscosity ([η]).The equation related to the radius of gyration at a given ionic concentration is:

$$R_g{}^2 = \alpha_s{}^2(LL_t)/3 \quad \text{with} \quad L_t = L_p + L_e \tag{24.4}$$

where the contour length L (proportional to M) is large compared with L_t; α_s is the excluded volume which contains a large contribution from the electrostatic interactions. Otherwise, it is necessary to use the development proposed for the θ-state by Benoit and Doty, if it is accepted that with an excess of salt the θ-conditions are approached [24]. The model of worm-like chain polyelectrolyte is given in detail by Reed [25a].

The local stiffness of polysaccharides explains the high viscosity obtained at a given molecular weight, compared with flexible polymers, as well as the relatively low sensitivity to the external salt concentration (since $L_p > L_e$ in many conditions). It was shown that the specific viscosity is directly related to the overlap parameter $C[\eta]$ at zero shear rate and expressed by the following relation:

$$\eta sp = C[\eta](1 + k_1(C[\eta]) + k_2(C[\eta])^2 + k_3(C[\eta])^3) \tag{24.5}$$

in which $k_1 = 0.4$, $k_2 = k_1/2!$; $k_3 = k_1/3!$ [25b]. This relation is very important because it allows one to predict that, for a given polymer, under given thermodynamic conditions, all the viscosity values for different polymer concentrations and molecular weights are going on the same curve, when plotted as a function of $C[\eta]$. The k_1 value represents the Huggins constant and for many perfectly water soluble polysaccharides it equals 0.4. The parameter L_p (which controls [η]) is obtained experimentally (when the ionic concentration of the eluent is known) from the curve $R_g(M)$ established from steric exclusion chromatography (SEC) experiments, using a multidetection equipment in which three detectors are on line, namely, a differential refractometer, a multiangle light scattering detector and a viscometer [9].

When an anionic polymer, such as an alginate, is mixed with a cationic polymer (like chitosan in acidic conditions), an electrostatic complex is formed, whose stability depends on the pH and the salt concentration [26]. The mechanism of complex formation was established following conductimetric measurements and can be expressed by the following equilibrium:

$$\text{----}COO^-Na^+ + Cl^- \, {}^+NH_3\text{----} \leftrightarrow \text{----}COO^- \, {}^+NH_3\text{----} + Na^+ + Cl^- \tag{24.6}$$

In stoichiometric conditions, the complex is usually insoluble and can be exploited to obtain fibres, films or capsules. The chitosan–alginate complex was examined for alginates with different molecular weights and M/G ratios [27, 28] given its many applications, particularly in the biomedical field.

24.3 CELLULOSE IONIC DERIVATIVES

24.3.1 Carboxymethylcelluloses

Cellulose ethers are obtained by the substitution of hydroxyl groups with ether groups. Different derivatives are commercially available, but the most important among the water soluble products are the CMC. These compounds, depending on the substituent density (or the degree of substitution, DS, characterizing the average number of

substituents per glucose unit) become soluble in water when the degree of carboxymethylation is larger than about 0.5. CMC is the most important cellulose ether, with a production of approximately 300 000 tons per year. It is produced by the reaction of alkali-cellulose with sodium chloroacetate or chloroacetic acid [29, 30]. We have produced, on a laboratory scale, soluble CMC with DS varying from 0.5 to 3. The typical raw materials for cellulose ether production are wood pulp or cotton linters, the latter being preferred for the production of high-viscosity ethers because of their higher degree of polymerization. The production of CMC can be carried out at atmospheric pressure, an advantage compared with the majority of the other cellulose ethers. In order to improve the diffusion of alkali and the etherifying reagent into the cellulose, inert solvents such as isopropyl alcohol or *t*-butyl alcohol are used. This leads to a more uniform substitution, and hence a higher water solubility. After etherification, the reaction mass or slurry may be neutralized with hydrochloric or acetic acid giving a crude CMC with a salt content (sodium glycolate or sodium chloride) of up to 40 per cent. If further purification is needed, these salts are extracted with water–alcohol mixtures (normally ethanol or methanol) before drying, grinding, screening and storage.

The CMC sodium salt is a white, odourless, hygroscopic and non-toxic solid. The DS of commercial samples may be between 0.3 and 1.2 (the majority of them have a DS between 0.65 and 0.85), although clear and fibre-free CMC solutions require a minimum DS value of about 0.5. Most CMC solutions are highly pseudoplastic and often show a thixotropic behaviour, which decreases when the macromolecules are more uniformly substituted. Recently, CMC were prepared from different non-wood fibres [31, 32]. Figure 24.2 shows the rheological behaviour of CMC from Abaca fibres after a first reaction giving a DS of 0.95 (ABE 1; $[\eta] = 601\,\mathrm{mL\,g^{-1}}$) and after a second reaction increasing it to 2.4 (ABE 2; $[\eta] = 410\,\mathrm{mL\,g^{-1}}$). At $100\,\mathrm{g\,L^{-1}}$, ABE 1 displayed a gel-like behaviour due to loose interchain interactions (which exist even at $30\,\mathrm{g\,L^{-1}}$), but ABE 2 had a viscoelastic behaviour at low frequency $G'' > G'$ [32a]. The rheological behaviour of CMC in the semi-dilute regime was also studied recently [32b].

The viscosity of aqueous solutions varies as a function of pH, showing a maximum at pH 6–7. It is sensitive to the added salt concentration which is explained by the polyelectrolyte properties of these water soluble derivatives. The role of the charge density on the solution properties (activity of counterions, pK etc.) was abundantly examined in our laboratory and will be briefly recalled here. The intrinsic pK of the water soluble CMCs in their acidic form, whatever their DS is, was found to be 3 ± 0.2 [33]. The activity coefficients of monovalent and divalent counterions depend directly on the DS and follow the theoretical prediction, as discussed in Reference [33]. Using CMC prepared in our laboratory and covering a wide range of DS up to 3, we demonstrated that an ionic selectivity among monovalent counterions occurred for DS $\geqslant 1$ with Li > Na > K > Cs > TMA. These important results were obtained by ultrasound absorption experiments, as shown in Fig. 24.3, where the acidic form was neutralized with different hydroxides. The limit for the appearance of ionic selectivity is in good agreement with the critical charge parameter $\lambda = 1$ introduced by Manning for the condensation [7]. This indicated that among the atmospheric counterions, a small fraction formed ion pairs.

CMC have good film forming properties, innocuity and an excellent behaviour as a protecting colloid and an adhesive, which make their field of applications very wide, including textiles, paper making, paints, drilling muds, detergents, foodstuffs (under the reference E466), cosmetics, pharmaceuticals and agricultural aids [3a].

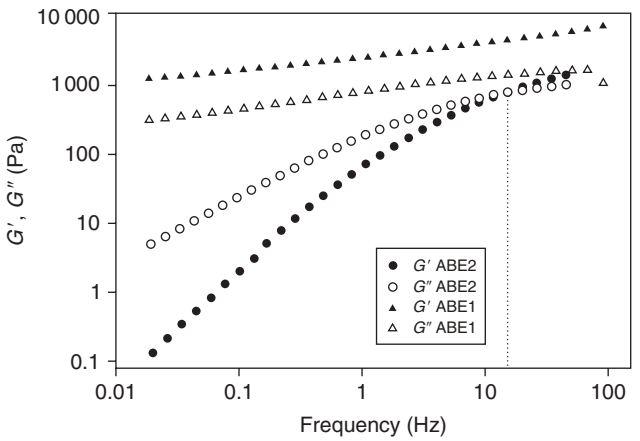

Figure 24.2 Storage and loss moduli as a function of frequency for $100\,\mathrm{g\,L^{-1}}$ abaca CMC samples isolated after one (ABE 1) and two treatments (ABE 2). (Reproduced with permission from Reference [32]).

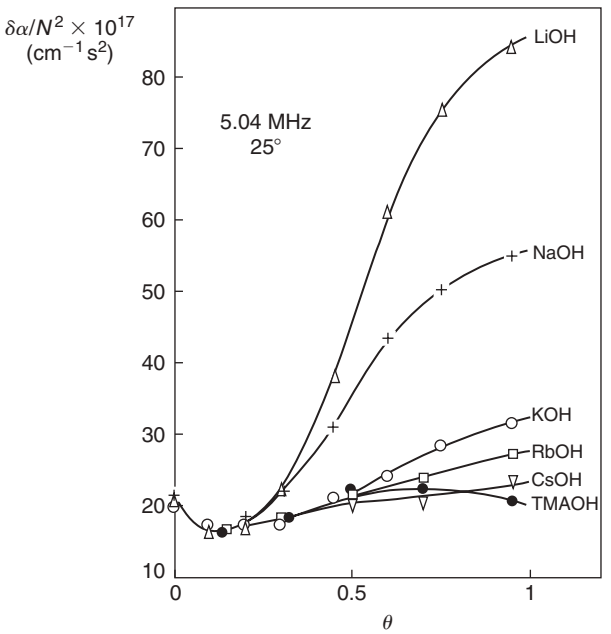

Figure 24.3 Ionic selectivity demonstrated by ultrasound absorption on CMC with a DS = 2.5, showing the role of the counterion. Tetramethylammonium counterion, TMA, is chosen as reference as it does not form ion pairs. (Reproduced with permission from Reference [18].)

CMC can be crosslinked with epichlorhydrine (in alkaline conditions) or formaldehyde (in acidic conditions) to give cation-exchange gels or ultrafiltration membranes [34, 35]. At low pH, CMC may form crosslinks through lactonization between carboxylic acid and free hydroxyl groups.

It has been shown recently that CMC adsorbs on cellulosic fibres improving the stabilization of their dispersion in an aqueous medium. This phenomenon was studied by electrokinetic measurements and rheology [36], and it was also demonstrated that these modified fibres can be dried and redispersed easily in a reversible way. Some of these results are given in Fig. 24.4 and show that the role of these additives is nearly independent of the CMC characteristics. Among different additives tested in this context, CMC were found as the most efficient.

Apart from (sodium) CMC, other mixed ethers exist in the market, such as carboxymethylhydroxyethylcellulose (CMHEC) used in drilling muds or completion fluids, and carboxymethylmethylcellulose (CMMC), used as a binder and as an adhesive for tobacco leaves.

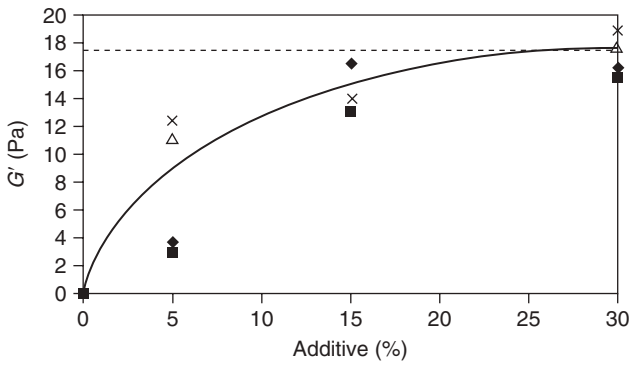

Figure 24.4 Storage modulus (G') at 0.17 Hz for the redispersion of a cellulose microfibril suspension (4 g L^{-1}) treated with CMC with different molecular weights and different DS. The dotted line is the initial never-dried suspension. ◆ CMC DS = 0.7 Mw = 1×10^6; 7HF; ■ CMC DS = 0.7 Mw = 3×10^4; 7ULC; Δ CMC DS = 1.2 Mw = 3×10^5; 12M8P; X CMC DS = 2 Mw = 6×10^5; X8212. (Reproduced with permission from Reference [36].)

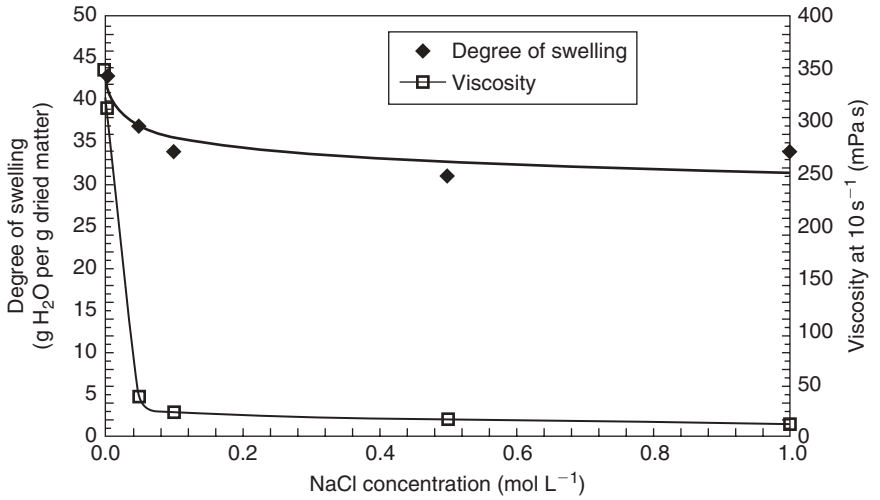

Figure 24.5 Degree of swelling of a suspension of cationic cellulose fibres (average DS = 0.45) and viscosity at $10\,s^{-1}$ in the presence of different external salt concentrations, at fibre concentration $C = 9.83\,g\,L^{-1}$. (Reproduced with permission from Reference [37].)

24.3.2 Cationic and carboxymethylated fibres

Heterogeneous polyelectrolyte systems can be prepared by mild chemical modifications of cellulose fibres; partially carboxymethylated pulps have been known to produce dispersible papers for a long time. More recently, we developed a technique to prepare cationic fibres with different degrees of substitution using cellulose from wheat bran, an agricultural residue [37]. The ensuing suspensions had a yield stress over $5\,g\,L^{-1}$, directly related to the DS and the fibre concentration; higher fibre concentrations gave a gel-like consistency. The degree of swelling and the viscosity of a suspension of cationic fibres depend directly on the salt concentration in tune with the behaviour of soluble polyelectrolytes (Fig. 24.5).

24.3.3 Other charged cellulose derivatives

Cellulose xanthate is produced as an intermediate in the viscose process (even though it is not generally used as a final product) used to prepare regenerated cellulose fibres. The progressively decreasing importance of this technology due to its high cost and impact on the environment, has reduced the interest in cellulose xanthate, which has been employed for the selective flocculation of minerals [38].

Phosphorylated derivatives are also available. Cellulose pulp or linters can react with phosphoric acid in a urea melt or with a mixture of phosphoric acid and phosphorus pentoxide in an alcoholic medium to prepare cellulose phosphate. Cellulose phosphites and phosphonates are produced via transesterification with alkyl phosphites. All these compounds have fire-retarding properties and ion-exchange properties and cellulose phosphate is used in textile and paper manufacture, as well as in the treatment of kidney stones.

Other derivatives include cellulose sulphates and borates, which however have not yet found industrial applications.

24.4 STARCH IONIC DERIVATIVES

24.4.1 Starch succinates

Succinic anhydride reacts directly with starch to form a half-ester called starch succinate. Starch can also react with alkyl or alkenyl derivatives of succinic anhydride to form the corresponding succinates, among which one of the most used is starch sodium octenylsuccinate. Due to the presence of hydrophobic and hydrophilic groups, this product has interfacial activity and emulsifying properties. Alkenylsuccinates are used in food, pharmaceutical and industrial applications [39].

24.4.2 Starch phosphates

Starch phosphates are derivatives obtained with phosphoric acid and include mono-, di- and tri-starch phosphate esters. The di- and tri-esters form crosslinked systems that contain also mono-phosphate. Monoesters can be produced by the reaction of starch with inorganic phosphates, with or without urea and with organic phosphorus-containing reagents. These products are anionic compounds that produce higher viscosity and more stable dispersions than unmodified starch. Their dispersions are very stable to freezing and because of their ionic properties, are good emulsifying agents. Starch phosphates are used as adhesives in papermaking, textiles, pharmaceuticals, foods, agriculture, flocculation and foundry [40]. Other ionic inorganic esters, such as starch sulphates, are used in foods, pharmaceuticals and petroleum recovery.

24.4.3 Cationic starch

Cationic starches are obtained by the reaction of starch with reagents containing amino, imino, ammonium or sulphonium groups. The two main types of commercial products are the tertiary amino and quaternary ammonium starch ethers. Among the reagents that can add quaternary ammonium groups to starch, probably the most popular is the 2,3-epoxypropyltrimethylammonium chloride. This reaction was examined in our laboratory where different DS were achieved on amylose and amylopectin separately and the products tested in calcium carbonate adsorption in relation with the mechanisms of dispersion and flocculation of small particles [41, 42]. The adsorption isotherm of amylopectins was investigated on water soluble polymers as a function of the DS and it was shown that the amount adsorbed decreased with increasing DS. This was related to the mechanism of adsorption: neutral amylopectin adsorbed forming loops and trains, but it adsorbed flat on the surface when its cationic DS increased; at the same time, the electrokinetic potential of the particles became highly positive and the particles were finely dispersed in the aqueous suspension [42]. Some relevant data are recalled in Fig. 24.6.

In some cases, cationization can be combined with other treatments, such as acid hydrolysis, oxidation or dextrinization to produce derivatives with a large range of viscosities. The key factor in the usefulness of these products is their affinity of negatively charged substrates. For that reason, cationic starches are used in papermaking (an example is the HI-CAT® cationic starch from Roquette, France). When they are used as a wet-end additive, the affinity between the positively charged cationic starch and the negatively charged cellulose fibres gives rise to an almost complete and irreversible absorption of starch. They are also used for filler retention in paper sheet formation and in other applications in textiles, flocculation, detergents, cosmetics and adhesives [43].

24.5 SEAWEED POLYSACCHARIDES

The cell walls of seaweeds contain polysaccharides which provide algae with their flexibility and help adapting them to the variety of water movements in which they grow. They also swell in water and thus preserve hydration. These polymers, often named phycocolloids, are usually extracted from the algae with water in different temperature conditions. The three main commercial polysaccharides are agars (including agarose, a neutral polysaccharide), alginates (carboxylic polysaccharides) and carrageenans (sulphated polysaccharides) and are used mainly for their thickening and gelling properties, depending on the thermodynamic conditions and on their molecular structures, as discussed below. Red seaweeds contain mainly agars and carrageenans, whereas brown algae produce alginates.

24.5.1 Alginates

Brown algae contain large amounts of anionic polysaccharides in their cell walls. Alginate, also named alginic acid or algin, was discovered in 1880. The quantity and quality of the alginates extracted depend on the algae and on the harvesting season. The total production of alginates, extracted mainly from the species of the orders *Laminariales* and *Fucales* is around 40 000 tons per year, of which 30 per cent is used in the food industry. They can be isolated under different ionic forms and applied in foods in the acid (E400), sodium (E401), potassium (E402), ammonium (E403) or calcium form (E404) (E is the code for food additives in the EU regulation).

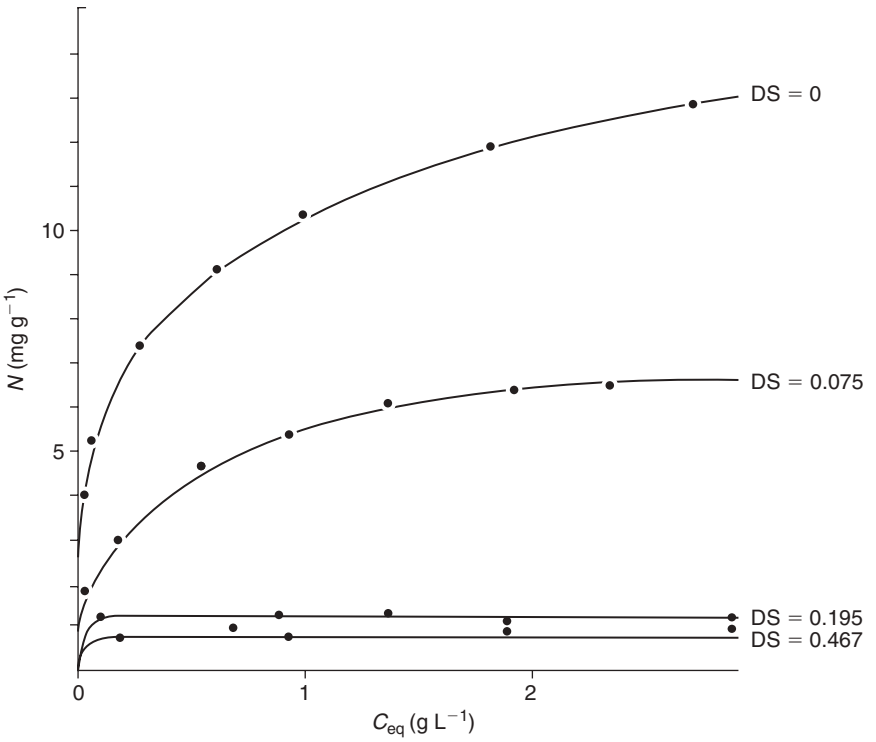

Figure 24.6 Adsorption isotherms for cationic amylopectins with different DS on calcite dispersed in water. $T = 25°C$. Amount adsorbed N expressed in milligrams per gram of solid; equilibrium polymer concentration expressed in grams per litre. (Reproduced with permission from Reference [41].)

Alginates are linear block copolymers composed of 1,4-linked β-D-mannuronic acid (M) with $^4C_1$ ring conformation and α-L-guluronic acid (G) with $^1C_4$ conformation, present in varying proportions and in their pyranosic structure. They are formed of three types of blocks: alternated M and G blocks, resistant to acid hydrolysis, the most flexible part of the chain, blocks of GG and of MM, with a DP $\geqslant 20$. In relation to their chemical structure, it was demonstrated that the physical properties of these polymers in aqueous media depends, not only on the M/G ratio, but also on the distribution of the M and G units along the chain. The GG blocks in which an axial–axial linkage is involved, are more rigid than the diequatorially linked MM blocks; hence, the stiffness of the chain, as well as its calcium complex formation, depends on the composition and distribution of the M and G units, as discussed by Smidsrod [44]. G blocks of more than 6–10 residues each, form stable crosslinked junctions (and gels) with divalent counterions (Ca, Ba, Sr), but not with Mg, as was also found with pectins with a low degree of methylation. It was also shown that the relative viscosity increases rapidly over a critical amount of divalent counterions (with Ca, Ba, Sr, but not with Mg) when the chains interact [45].

At low pH, alginates form acidic gels stabilized by H-bonds. The homopolymeric G blocks form the junctions and the stability of the gels is determined by the relative content and length of the G blocks.

For their characterization, alginates must first be purified and isolated under their sodium form. NMR spectroscopy (^{1}H and ^{13}C) is the most powerful technique to characterize the chemical composition and the microstructure of alginates [46–49]. Purified alginates, isolated under the sodium salt form, were also characterized by steric exclusion chromatography (SEC)with three detectors on line. For commercial products, molecular weights may range between 32 000 and 400 000. A further means of characterization is their intrinsic viscosity using the Mark–Houwink relation:

$$[\eta](\text{mL g}^{-1}) = KM^a \tag{24.7}$$

with $K = 2 \times 10^{-3}$ and $a = 0.97$, in a 0.1 M NaCl aqueous solution at 25°C [50].

The dimensions of the alginate chains (radius of gyration and intrinsic viscosity) in aqueous solutions depend on the external salt concentration, their expansion being directly related to the thickening performance of the polymer. It seems, however, that the expansion of polysaccharides, even in external salt excess, is larger than that of a random coil in the absence of electrostatic repulsion, because of the semi-rigid character usually associated with this type of polymers [9]. The relative extension of the three types of blocks in alginates increases in the following order [51]:

$$MG < MM < GG$$

which is in agreement with the L_p values discussed above [23].

The most important technical properties of alginates are their thickening character (increase in the solvent viscosity upon dissolution), their ionic exchange aptitude, and their gel-forming ability in the presence of multivalent counterions. These features are a direct consequence of the fact that alginates are polyelectrolytes and follow, therefore, the usual behaviour of charged polymers. Alginates, in their monovalent salt form, are water soluble whatever be the temperature and their ionic properties (electrostatic short- and long-range interactions), as well as their conformation and molecular weight, are important factors that control their rheological behaviour. The viscosity of their aqueous solutions depends on their concentration, molecular weight and external salt concentration (because of the screening effect of electrostatic interactions). The viscosity of alginate solutions is nearly constant between pH 6 and 8 but, at moderate concentrations, it increases below pH 4.5 and reaches a maximum around 3–3.5 and then decreases (alginic acid forms gels). This behaviour arises from the fact that H-bond attractions dominate over electrostatic repulsions, as was observed recently with hyaluronan [52]. In dilute solutions, the viscosity decreases when pH decreases from pH $\sim$ 6 and in the range of pH 1–4, alginic acid is hydrolyzed. These solutions are considered to be stable in the range of pH 5–10. The intrinsic pK of alginic acid is about 3, as is the case for many polyuronic acids [53].

Another characteristic of alginates, in addition to their gelling and stabilizing properties, is their ability to retain water. Because of their linear structure and high molecular weight, alginates also form strong films and good fibres in the solid state.

The mechanism of gelation is related to the chemical structure of these polymers. First, electrostatic properties are important for the selective interactions with divalent counterions. A specific cooperative Ca interaction forms on G blocks, which is the basis of the junction zones and the crosslink of an ionic network, as soon as the degree of polymerization is larger than 20 (Fig. 24.7); this interaction is not observed for M unit blocks [54, 55].

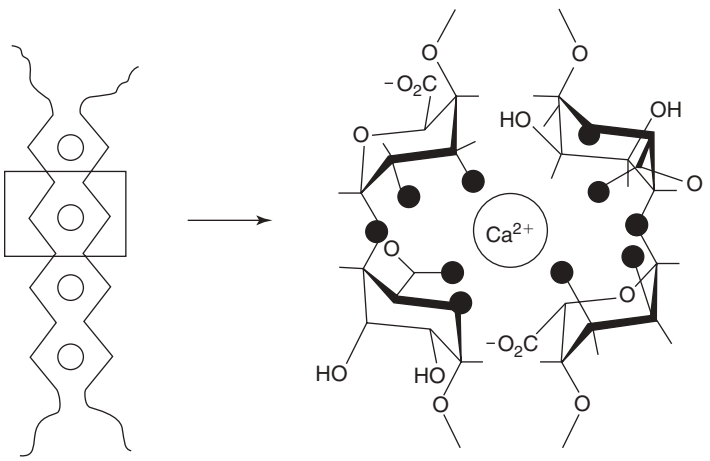

Figure 24.7 Specific interaction of calcium ion with α-L-guluronic-box block. Dark circles represent the oxygen atoms involved in the coordination of the calcium ion. (Reproduced from Reference [57] with the permission of ACS.)

The conformation of acidic polysaccharides and their interactions with calcium ions was examined by molecular simulation, and the authors demonstrated the existence of specific calcium binding with poly-α-L-guluronate [56, 57]. The mechanism of complex formation involves calcium interactions with different oxygen atoms of two adjacent guluronic acid units and with two inter-chain units, as visualized in the egg-box model (Fig. 24.7). The mechanism of gelation is a two-step process: first step is a dimer formation, followed by precipitation for small chains, or gelation for long ones formed with different types of blocks.

The properties of the ensuing gels depend on the molecular characteristics of the alginates; the stability of the gels and their physical properties depend directly on their G content and on the length of their G blocks. It is clear that the stiffness of the gels increases when the G content and the length of the G blocks increase [58]. Figure 24.8 shows that the gel strength increases for a given molar structure when the molecular weight increases up to a limit around $M = 3 \times 10^5$, a behaviour similar that of κ-carrageenan [59].

Alginates have interesting ion-exchange properties: most monovalent counterions (except Ag^+) form soluble alginate salts, whereas divalent and multivalent cations (except Mg^{2+}) form gels or precipitates. The affinity was found to follow the order [60–63]:

$$Mn < Zn,Co,Ni < Ca < Sr < Ba < Cd < Cu < Pb$$

The amount of salt required to induce gelation increased in the order [64, 65]:

$$Ba < Pb < Cu < Sr < Cd < Ca < Zn < Ni < Co < Mn,Fe < Mg$$

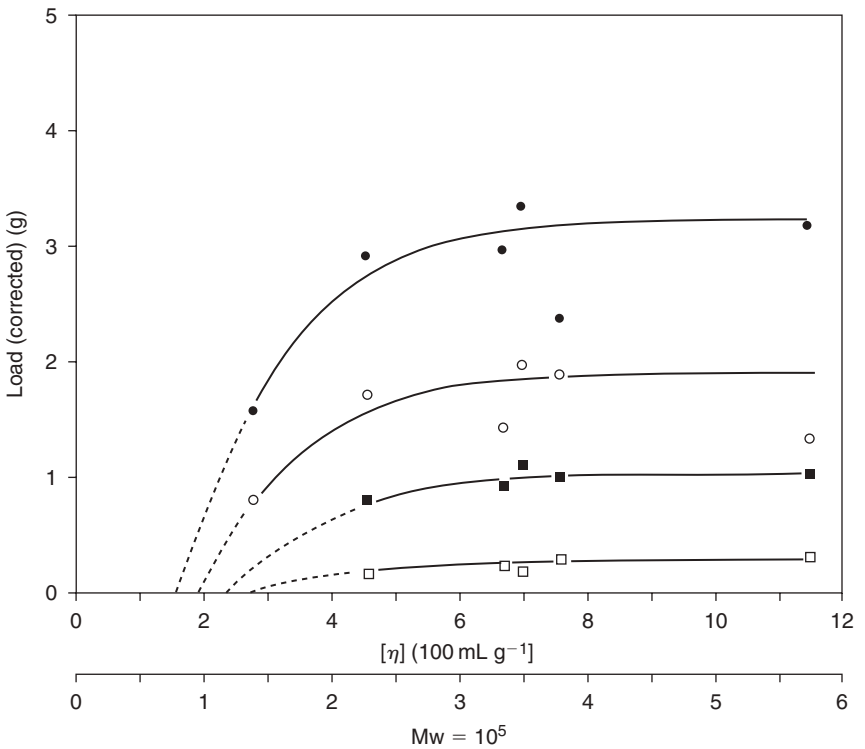

Figure 24.8 Influence of the molar mass, intrinsic viscosity and polymer concentration on the gel strength of alginate from *L. hyperborea* (outer cortex) $F_G = 0.75$, $N_{G>1} = 17.5$. Concentration: (•) 1 per cent, (○) 0.8 per cent, (■) 0.6 per cent and (□) 0.4 per cent. (Reproduced with permission from Reference [58].)

The efficiency of a divalent ion as a precipitant for alginate depends, not only on its affinity for the alginate, but also on the amount of ions which must be bound to the alginate for gel formation. This sequence is not exactly the same as the one given above.

Purified alginates are produced under different salt forms: Na, K, NH_4, Ca and/or propylene glycol derivative as food additives for thickening soups and jellies. They are used as anti-acid preparation such as Gaviscon®, in mould-making materials in dentistry in the presence of a slow release calcium salt to control the gelation delay. Alginates in the form of fibres or films are commercialized as haemostatic materials and wound dressings, like AlgiDERM® or Sorbsan® made of sterile purified calcium alginate fibres. Calcium-based gels are often used in a bead form as immobilization matrices for animal cells or plant protoplast [66–68]. Finally, polyelectrolyte complexes are often used in biomedical applications; thus, chitosan–alginate beads were evaluated for tissue engineering [69] and wound dressing [70].

24.5.2 Carrageenans

Agars and carrageenans are the most important polymers extracted from Red algae. Many Rhodophycean galactans, with a few exceptions, have a linear chain structure of β-D-galactopyranose residues linked through positions 1 and 3 (A units) and α-galactopyranose residues linked through positions 1 and 4 (B units) arranged in an alternated $(AB)_n$ sequence. Units A may carry methyl ether groups at position 6, sulphate hemiester groups at position 2, 4 or 6 and a few A units may carry pyruvic acid, linked as a cyclic ketal, bridging *O*-4 and *O*-6 (1-carboxyethylidene groups). Units B can occur in either D or L form, can carry methyl groups at position 2, 4-*O*-methyl-α-L-galactopyranosyl groups at position 6 and sulphate hemiester groups at position 2 or 6, or both; B units can be wholly or partly converted into 3,6-anhydro forms by the elimination of the sulphate moiety from position 6 (by enzymatic elimination or alkaline treatment) [71]. The ideal composition of the polysaccharides obtained depends on the species, habitat, harvesting season and on the extraction conditions.

Carrageenans belong to two families: the κ-family includes ι- and κ-carrageenans, which contain as B unit a 3,6-anhydro-D-galactose, forming gels in the presence of K^+ counterions (see below), as well as the μ- and ν-carrageenans, without the anhydrogalactose, also named the precursors; the λ-family includes λ- and ξ-carrageenans with no anhydrogalactose, nor gelling properties [71].

The main physical properties of these three carrageenans are:

- Kappa (κ) → strong and rigid gels (polysaccharides extracted from *Kappaphycus cottonii, Chondrus, Hypnea, Furcellaria*).
- Iota (ι) → soft gels (polysaccharides extracted from *Euchema spinosum, Hypnea, Gigartina*).
- Lambda (λ) → thickening polymer (polysaccharides extracted from *Gigartina pistillata, Chondrus crispus*).

All of them are soluble in hot water but form gels on cooling, except the lambda type which remains water soluble at low temperature. Emphasis will be placed below on the properties and characterization of ι- and κ-carrageenans.

^{1}H NMR is the most powerful technique available to identify the type of carrageenan, but it requires the purification of the samples, preferably under the sodium salt form, to be sure that the polymer is in the coiled conformation in dilute solution, even at ambient temperature. This technique also gives rapid access to the quantitative determination of the different substituents, if any, and to get information on the purity of the sample tested. This point is important because the chemical structure directly controls the physical properties of the polysaccharide.

The application of steric exclusion chromatography was again adopted to determine the molecular weight distribution. For κ-carrageenan at 25°C in 0.1 M NaCl, the intrinsic viscosity gives access to the molecular weight, using the following relation, obtained from fractions isolated by preparative gel chromatography [72]:

$$[\eta](\mathrm{mL\,g^{-1}}) = 3.1\times10^{-3}M^{0.95} \tag{24.8}$$

For stereoregular charged polysaccharides, the formation of a helical conformation and eventually gelation depend on the ionic concentration, the nature of electrolyte and the temperature. The conformation results from a balance between H-bonds (which stabilize the helical conformation, but which are destabilized when the temperature increases) and electrostatic repulsions between the charges on the polymer (which are screened by external salt addition). The helix–coil transition for κ-carrageenan was demonstrated by different techniques, viz. conductivity,

optical rotation, NMR and DSC [73–75]. Optical rotation measurements showed that an ordered conformation is stabilized at low temperature and goes to a disordered conformation when the temperature is increased. This conformational transition is characterized by T_m, the temperature for conformational helix–coil change, determined at half transition. In fact the helix–coil transition is perfectly reversible (no hysteresis) at low ionic concentration (and low polymer concentration playing the role of electrolyte), but hysteresis appears when the ionic concentration increases [76]. The hysteresis is modified after ageing because of an increase in the degree of aggregation, which is in fact related to a synaeresis phenomenon. At the same time, at lower temperatures, the molecular weight is doubled and the activity coefficient γ of monovalent counterions decreases. At 15°C and 35°C, the activity of Na^+ is practically not influenced by neither temperature nor polymer concentration and tends to 0.71 at infinite dilution [76, 77]. This value is in good agreement with the prediction from polyelectrolyte theories and corresponds to a single linear chain. The same values are obtained for Na^+ and K^+ at 35°C, but at 15°C, a transition is observed when the polymer concentration increases and the K^+ activity coefficient goes to 0.37, corresponding to a double charge parameter λ, indicating the association of two chains to form a double helix (as corroborated by optical rotation) [76]. This behaviour also demonstrates the existence of an ionic selectivity, as discussed below. A doubling of the molecular weight of ι-carrageenan was also found in non-aggregating conditions [77].

The different values of T_m (temperature for conformational change), T_F (melting temperature) and T_G (gelling temperature) were determined from heating and cooling curves obtained by optical rotation or DSC of aqueous solutions of κ-carrageenan and plotted in a log–log representation of these characteristic temperatures (T^{-1}) as a function of the total ionic concentration C_T (taking into account the polyelectrolyte contribution, γC_p, C_p being the polyelectrolyte concentration expressed in charge equivalents per litre). The helix–coil transition is perfectly reversible (no hysteresis) at low ionic concentration (C_T lower than a critical value C_T* around 7.5×10^{-3}M in KCl and 2×10^{-1}M in NaCl), but hysteresis appears when the ionic concentration increases ($T_G \sim T_m < T_F$). Figure 24.9 shows the phase diagram of κ-carrageenan in the presence of K^+ and Na^+ counterions and salt excess [78].

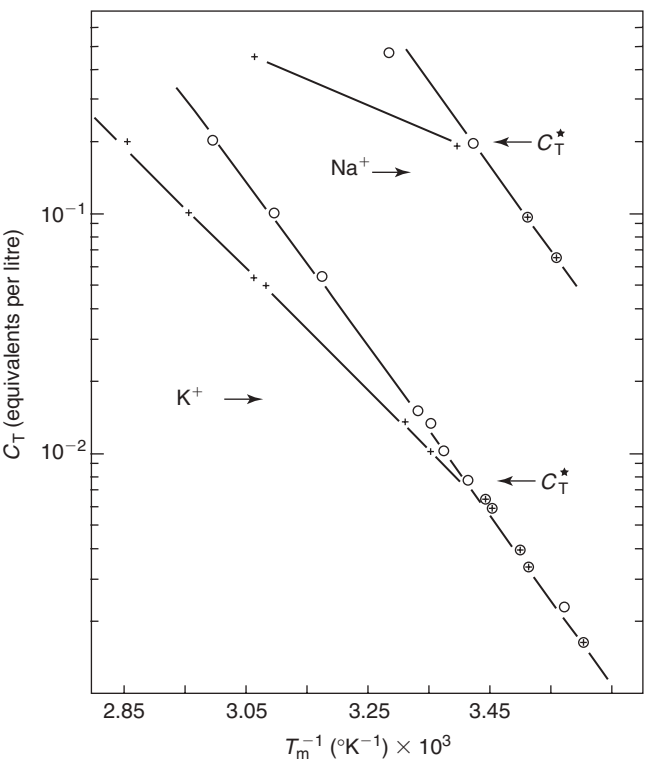

Figure 24.9 Phase diagram for κ-carrageenan in K- and Na-salt forms. Inverse of the transition temperature (T_m) as a function of the total ionic concentration (C_T). T_m (+,○) when $C_T < C_T$* and T_F (+) and T_G (○) when $C_T > C_T$* with the logarithm of the total concentration of counterions C_T (including external salt and free counterions from the polymer). (Reproduced from Reference [78] with the permission of ACS.)

This figure gives evidence of ionic selectivity, which appears in the coil conformation first and is associated with ion pair formation, as demonstrated using ultrasound absorption [17]. The ion pair formation with K^+ reduces the net charge of the polymer and favours the helical dimer formation.

For κ-carrageenan, the ionic selectivity among monovalent cations is very high whereas it is low among divalent counterions [76]. The T_m values were determined at a constant ionic concentration (0.1 M) and T_m in the presence of monovalent counterions varied according to the following sequence:

$$Rb^+ > K^+, Cs^+ > Na^+ > Li^+, NH_4{}^+ > R_4N^+$$

No selectivity was observed among the monovalent anions with the exception of I^-, which stabilizes the helix, but prevents gelation [76, 79].

Agarose, κ-carrageenans and ι-carrageenans are recognized as gelling polymers, but their specific properties depend on their chemical structure; the conditions of gelation, as well as its mechanism, have been abundantly discussed in the literature [80]. The physical thermoreversible gel formation is based on the establishment of H-bonds between double helices which associate in aggregates (or junction zones) giving rise to a three-dimensional network. It has been shown that gelation proceeds following a two-step process, as clearly demonstrated for thermodynamic conditions around C_T* and a polymer concentration larger than its overlap concentration. The width of the hysteresis in temperature is directly related to the degree of aggregation of the double helices and to the charge density of the polymers (this width decreases from agarose to κ-carrageenan to ι-carrageenan). The related rigidity of the gels formed in the presence of K^+ counterions follows the same trend and varies in the following order:

$$\text{agarose} > \kappa\text{-carrageenan} > \iota\text{-carrageenan}$$

(even if the salt effect is not the same for agarose).

The mechanical properties of the gels formed in the presence of different counterions follow the same order as that of the stability of the double helices: potassium-κ-carrageenan gel forms at lower polymer and ionic concentrations and has a higher modulus and a higher melting temperature than the corresponding sodium-κ-carrageenan gel, which forms at a much higher ionic concentration and has a lower modulus. It was shown that ι-carrageenan displays only a very low ionic selectivity [81].

The mechanical properties of the κ-carrageenan gels were investigated by compression measurements (Fig. 24.10) and it was demonstrated that the elastic modulus, when the gels were formed at a given KCl concentration, increased when the molecular weight increased up to $M \sim 250\,000$ and that the modulus was directly related to the polymer concentration. For the same polymer concentration, the modulus was found to depend not only on the molecular weight, but also on the ionic KCl concentration (causing the screening of the electrostatic repulsion) and went to a limit for the same range of molecular weights [59]. Conversely, the yield stress for gel rupture increased linearly with molecular weight in the range covered [82].

The gels from ι-carrageenan are less strong and have less synaeresis and their properties often depend on the presence of κ impurities. Their elastic modulus increases when the ionic concentration increases up to 0.25 M and decreases for higher concentrations due to a salting-out effect; thus, a $10\,g\,L^{-1}$ gel of ι-carrageenan formed in 0.25 M KCl has an elastic modulus of 0.32×10^4 Pa, while for a pure κ-carrageenan counterpart in 0.25 M KCl it is 6.6×10^4 Pa. To conclude, the stiffness of ι-carrageenan gels is low because of shorter junction zones with lower stability (larger charge density) and they shrink strongly in non-solvents [83].

A general relationship was found to apply to all these physical gels, in 0.25 M KCl, in terms of the variation of their elastic modulus, viz:

$$E\,(\text{Pa}) = K\,C^{2\pm0.1} \tag{24.9}$$

in which C is expressed in grams per litre and $K = 745$ and 43 for κ- and ι-carrageenans, respectively. Comparative data for different polysaccharide gels are also available [84].

For industrial applications, 30 000 tons per year of the three types of carrageenans are produced. When used for food products, carrageenans have the EU additive number E407. Their essential characteristics make them ideal for

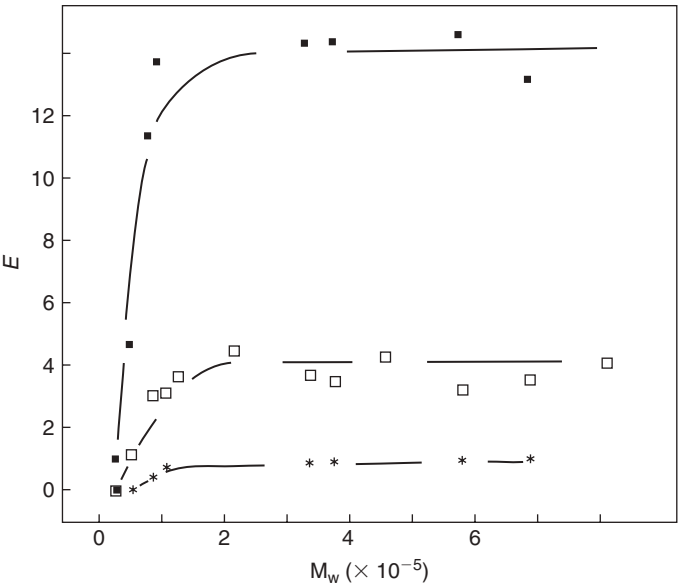

Figure 24.10 Elastic modulus (10^4 Pa) obtained in compression measurements, as a function of the molecular weight of different fractions of κ-carrageenan in 0.1 M KCl for 5 g L^{-1} (*); 10 g L^{-1} (□); 20 g L^{-1} (■). (Reproduced with permission from Reference [59].)

use as thickening and stabilizing agents. Other applications include the solidification of flans, yoghurts, chocolate milk, ice creams and milk puddings and the solidification and emulsification of solutions, the prevention of chocolate milk from creaming and sedimenting. Toothpastes and canned and frozen petfoods also contain carrageenans to promote solidification. Beers are clarified with carrageenans used to complex and precipitate proteinaceous impurities. As agarose and alginates, carrageenans are used for encapsulation. The specific features brought about by these incorporations of carrageenans depend on the composition of the systems into which they are introduced.

24.6 PECTINS

Pectins are located in the middle lamella and primary cell walls of plant tissues and consist primarily of 1,4-linked α-D-galacturonic units or their methyl esters with some interruptions by rhamnogalacturonan region kinking the linear polygalacturonic backbone [85]. They also contain branched chains composed of neutral sugars such as galactose or arabinose. These neutral sugars amount to 10–15 per cent of the pectic dried weight and are concentrated in blocks (called the hairy region) [86]. Pectins are mostly extracted from apple marks and citrus peels. Pectins with different degrees of esterification (DE) represent about 70 per cent of all natural structures, depending on the age of the source and on the presence of enzymes (causing blockwise or random distribution of free carboxylic groups in relation with the nature of the enzyme); if DE $<$ 50 per cent, they are insolubilized by calcium association in the plant and extraction will impose the use of chelating agents, such as sodium oxalate. Two categories of these pectins are recognized: HM pectins (high methoxyl; DE $>$ 50 per cent) form gels in acid conditions and in the presence of sucrose to decrease the water activity. The gelation is based on H-bond association and is thermoreversible [87]. LM pectins (low methoxyl; DE $<$ 50 per cent) form gels in the presence of calcium ions [88]. HM pectins can be extracted by water, mineral acids or bases. These differences are shown in Fig. 24.11, where the activity coefficient of calcium is determined on pectins with different DE (random distribution of the carboxylic groups). Aggregation of chains in the dilute regime occurs when DE $<$ 50 per cent [89].

LM pectins are immobilized *in situ* via metallic ions and need a sequestering agent to displace the counterions. Different treatments were compared between sugar beet and potato pulps [90, 91]. Before pectin extraction, a pretreatment of the plant material is necessary to inactivate enzymes (water at 85°C, 20 min). HM pectins naturally occur in sugar beet pulp and they were extracted in alkaline conditions (50 mM NaOH, pH 12) and precipitated with ethanol after neutralization to pH 6.5–7. On the contrary, for potato pulp containing an LM pectin, the

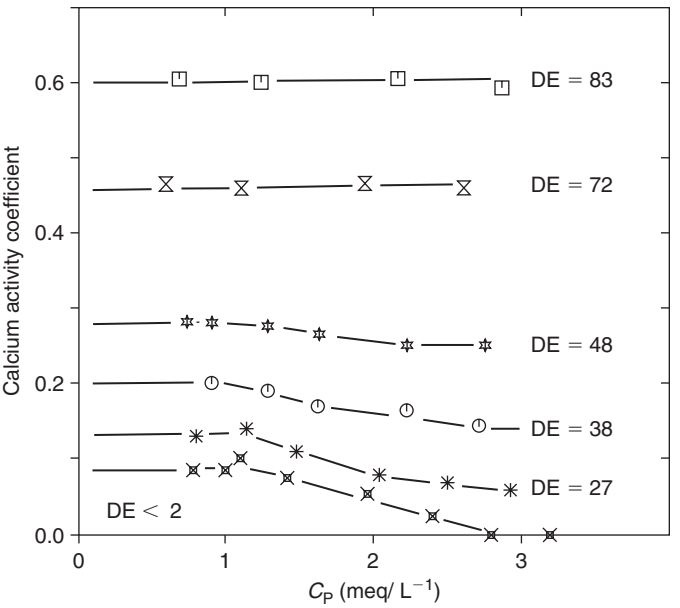

Figure 24.11 Calcium activity coefficient as a function of polymer concentration for different pectins. The DE are expressed in group per cent and vary from less than 2 to 83 per cent. (Reproduced with permission from Reference [89].)

extraction was performed in acidic conditions (pH 3.5 in the presence of 0.75 per cent hexametaphosphate, 75°C, 1 h). The pectins were precipitated at pH 2 with HCl. The precipitate was redispersed in water with NaOH up to pH 6.5–7 and reprecipitated with ethanol. Potato pulp was also extracted in alkaline conditions with hexametaphosphate, precipitated at pH 2, redispersed in water to pH 6.5–7 and precipitated with ethanol. The LM pectins obtained in alkaline conditions had a higher gelling ability because of deesterification and partial deacetylation. The alkaline treatment also has a fundamental role in the deesterification and produces a random hydrolysis of the ester groups. For both sources, pectins with good gelling properties in the presence of calcium ions were obtained.

The interaction with calcium is very cooperative due to the stereochemistry of the 1,4-linked monomeric galacturonic units, leading to the formation of polar cavity that can be occupied by calcium or related cations. The mechanism of interaction is described by the egg-box model already discussed above for alginates (see Fig. 24.7). A series of galacturonic acid oligomers were investigated to test the minimum carboxylic group concentration necessary to get a stable junction. A two-step process leads to gelation: first, a dimer formation occurs for DP $>$ 10 (or 5 Ca^{2+}), followed by aggregation forming the junction zones [53]. The influence of the uronic acid configuration was investigated by Kohn [54], who showed that specific interactions of Ca^{2+} occur with L-guluronic and D-galacturonic acid units, but not with D-mannuronic acid counterparts.

The ability to gel can be determined from the dependence of the viscosity or light scattering on the concentration of added counterions. At a given polymer concentration, the critical amount of cations at the gel point is directly related to the degree of esterification, but also to the distribution of carboxyl groups along the chain [92]. This is shown in Fig. 24.12, where two samples with the same average DE, but one bearing a blockwise distribution of carboxylic groups (a) and the other a random distribution (b) are shown.

It was also shown that the divalent counterions form gels with the affinity sequence Ba $>$ Sr $>$ Ca, but Mg does not form gels nor dimers [89, 93, 94]. This association was investigated by conductimetry, from which the transport coefficient (*f*, which is close to the activity coefficient) of the divalent counterions was determined on dilute solutions, as given in Table 24.2, where the experimental values are compared with the theoretical transport coefficients calculated from the Manning theory. These data explain, on the basis of the charge parameter, why, in the presence of Mg, the pectin remains as a single chain, whereas with Ba and Sr, the values are much lower, especially when DE $<$ 50 per cent, indicating dimer formation with some additional aggregation [89].

As mentioned above [26], LM pectins form polyelectrolyte complexes with chitosan, which were envisaged as encapsulating materials for shark liver oil [95].

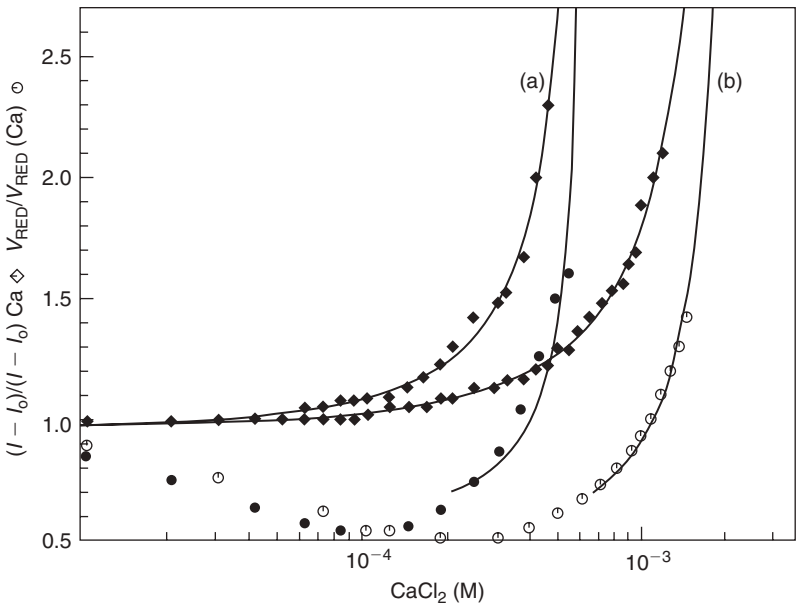

Figure 24.12 Changes in reduced viscosity (○, ●) and scattered light (◆) during the addition of $CaCl_2$ to pectin solutions (polymer concentration: $0.2\,g\,L^{-1}$; 5×10^{-3} M $CaCl_2$). Role of the carboxyl group distribution: (a) DE = 30 per cent blockwise distribution, obtained by pectin-esterase hydrolysis; (b) DE = 30 per cent random distribution, obtained by alkaline hydrolysis. (Reproduced from Reference [92], with permission from John Wiley & Sons, Inc., on behalf of the Society of Chemical Industry.)

Table 24.2

Transport parameters (f) of divalent counterions for pectins with different DE. Comparison of experimental values with the theoretical one calculated with Manning's theory [92].

DE (%)	λ	f for Ba^{+2}	f for Sr^{+2}	f for Mg^{+2}	Theoretical f
<2	1.58	0.095	0.110	0.280	0.276
21.4	1.26	0.140	0.171	0.290	0.344
72.1	0.45	0.475	0.529	0.630	0.889

24.7 GALACTOMANNAN IONIC DERIVATIVES

Galactomannans are neutral polysaccharides isolated from seeds (carob, guar, locust bean, tara, etc.). Their main chain is made of [(1 → 4)-β-D-Man] (Man = mannose; M units) with different degrees of substitution on *O*-6 with α-D-galactopyranosyl units (G). Their composition (or M/G ratio) is easily determined by ^{1}H NMR.

The solubility of galactomannans depends on the M/G ratio and on the distribution of galactose units along the mannan chain, the larger being the galactose content, the higher the solubility in water. The M/G ratio varies from 1/1 (in Mimosa scrabella) to 1/5 (in locust bean gum) and the rheological properties of galactomannan solutions depend on this ratio, because of the increase in loose interchain interactions when the G content decreases [96]. Molecular modelling was applied to the determination of the persistence length of a galactomannan model in which M/G ≈ 1 and a good agreement was obtained between experimental SEC determinations and the corresponding calculated; L_p was found in the range of 9.5 nm [97].

Although the water solubility of galactomannans is relatively low, the ensuing solutions display interesting rheological properties as thickeners. They have been modified by the specific oxidation at C-6 position with TEMPO in order to increase their solubility by transforming them into new polyelectrolytes [98, 99].

24.8 LIGNINS AND LIGNOSULPHONATES

Although lignins are not polysaccharides, a brief mention of their interest when they are in a polyelectrolyte form seems justified to conclude this chapter.

Lignins extracted from wood have modest polyelectrolyte behaviour and are usually soluble in alkaline media thanks to the presence of phenolic and a few carboxylic groups. A birch lignin extracted by 40 wt% sodium benzoate at 150°C displayed a net charge of about 3×10^{-3} equivalents per gram of lignin [100].

Lignosulphonate is a by-product of the sulphite process in the manufacturing of pulp (see Chapter 10). It is a complex mixture of small-to-moderate-size polymers and oligomers bearing sulphonate groups, which display a strong polyelectrolyte character and are often used to deflocculate clay-based muds.

24.9 CONCLUSION

The impressive variety of polysaccharide structures which display polyelectrolyte properties can be therefore exploited in a wide range of applications, those associated with human consumption and health care being of particular interest. Renewable resources play therefore a particularly useful role in providing readily available and often cheap macromolecular materials which are rarely matched by equivalent counterparts derived from fossil or inorganic sources.

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– 25 –

Chitin and Chitosan: Major Sources, Properties and Applications

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ABSTRACT

Chitin is widely distributed in nature, constituting an important renewable resource. The main sources of chitin generally used are the crustacean wastes of the fishing industry. The chapter gives a brief account of the main processes employed in chitin isolation and the preparation of chitosan by extensive deacetylation of chitin. The common methods of characterization of chitin and chitosan, in terms of degree of acetylation and molecular weight, are discussed. Their crystalline structure and their solution properties, are also described. The capacity of chitin and chitosan of forming complexes with metal ions is shown, and mention is made to some of its diverse applications. The ability of chitosan to form polyelectrolyte complexes with polyanions, the cooperativity of this reaction and the properties of chitosan-based polyelectrolyte complex membranes, are also examined. The chapter ends with a review of the applications of chitin and chitosan in medicine, pharmacy, agriculture, the food industry, cosmetics, among others.

Keywords

Chitin, Chitosan, Demineralization, Deproteinization, Deacetylation, Degree of acetylation, Metal ions complexation, Polyelectrolyte complex, Biomaterial, Flocculation, Antimicrobial activity

25.1 INTRODUCTION

Chitin is the second most abundant polysaccharide in nature after cellulose. It is widely distributed in the animal and vegetal kingdom, constituting an important renewable resource. It was first isolated from fungi by Braconnot in 1811 [1], but the name chitin – from the Greek $\chi\iota\tau\omega\nu$ which means tunic or cover – was given by Odier, who in 1923 isolated it from the elytrum of the cock-chafer beetle by treatment with hot alkaline solutions [2]. Because of its insolubility in the vast majority of common solvents, chitin was considered an intractable polymer and for many years it remained mainly a laboratory curiosity. However, as will be shown below, at present chitin and its derivatives have become polymers of great interest in a large variety of areas of human activity.

Chitin is generally represented as a linear polysaccharide composed of $\beta(1 \rightarrow 4)$ linked units of *N*-acetyl-2-amino-2-deoxy-D-glucose (Fig. 25.1(a)). Although it has been proposed that, depending on the source, a variable, but always small, proportion of these structural units are deacetylated in natural chitin, this has never been proved unambiguously, with recent evidence pointing to the contrary.

The great structural similarity existing between chitin and cellulose is shown in Fig. 25.1. The difference between them consists in that the hydroxyl group of carbon C2 in cellulose (Fig. 25.1(b)) is substituted by an acetamide group in chitin. Both biopolymers play similar roles, since they both act as structural support and defence materials in living organisms.

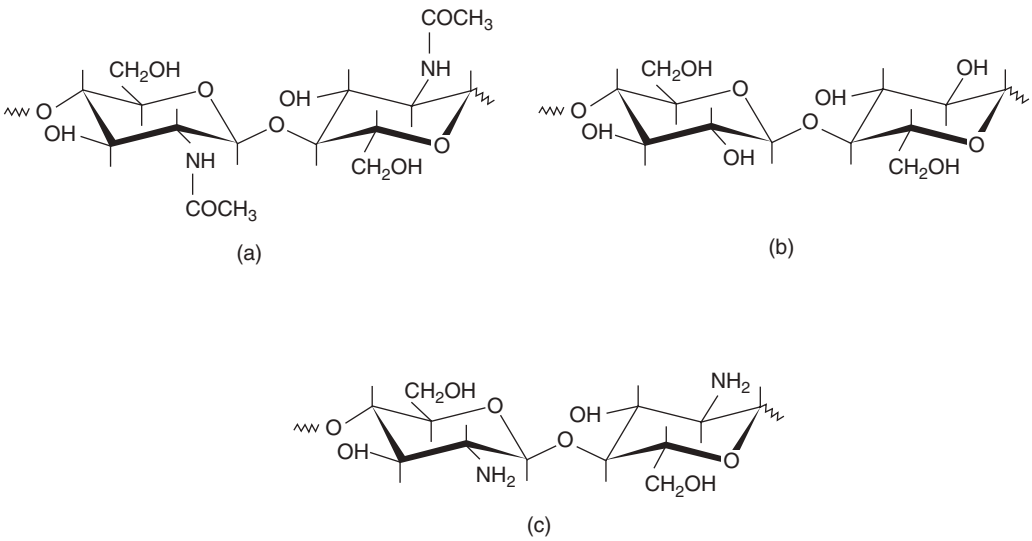

Figure 25.1 Schematic representation of (a) completely acetylated chitin; (b) cellulose and (c) completely deacetylated chitosan. The structural similarity between them becomes evident.

Chitosan is a linear polysaccharide obtained by extensive deacetylation of chitin. It is mainly composed of two kinds of β(1 → 4) linked structural units *viz*. 2-amino-2-deoxy-D-glucose and *N*-acetyl-2-amino-2-deoxy-D-glucose. The chemical structure of a completely deacetylated chitosan is represented in Fig. 25.1(c). However, since it is virtually impossible to completely deacetylate chitin, what is usually known as chitosan is a family of chitins with different but always low degrees of acetylation. The capacity of chitosan to dissolve in dilute aqueous solutions is the commonly accepted criterion to differentiate it from chitin.

Chitin is the most abundant organic component of the skeletal structure of many classes comprising the group of invertebrates, such as arthropods, mollusks and annelids. In animals, chitin occurs associated with other constituents, such as lipids, calcium carbonate, proteins and pigments. It has been estimated that the crustacean chitin present in the sea amounts to 1560 million tons [3]. Chitin is also found as a major polymeric constituent of the cell wall of fungi and algae. Fungal chitin exhibits some advantages as compared with animal chitin, such as a greater uniformity in composition, a continuous availability in time and the absence of inorganic salts in its matrix. However, in fungi chitin is associated with other polysaccharides, such as cellulose, glucan, mannan and polygalactosamine, which makes its isolation difficult [4].

Chitosan is also present in significant quantities in some fungi, such as *Mucor rouxii* (30 per cent) and *Choanephora cucurbitarum* (28 per cent), although again associated with other polysaccharides.

In order to improve the properties of these unique polysaccharides and to develop new advanced materials, much attention has been paid to their chemical modification. These polymers have two reactive groups suitable for this purpose, namely, primary (C6) and secondary (C3) hydroxyl groups in the case of chitin whereas chitosan has additionally the amino (C2) group on each deacetylated unit. All these functions are susceptible to a variety of classical reactions which can be applied here in a controlled fashion to obtain a vast array of novel materials based on the two polysaccharides which can also be modified by either crosslinking or graft copolymerization. This topic has been extensively studied and thoroughly documented [5–7].

25.2 METHODS OF PREPARATION

25.2.1 Isolation of chitin

Commercial chitin is extracted from crustacean wastes of the fishing industry, the main chitin sources being the shells of shrimp, crab, lobster, prawn and krill. These crustacean wastes consist of chitin (20–30 per cent), protein

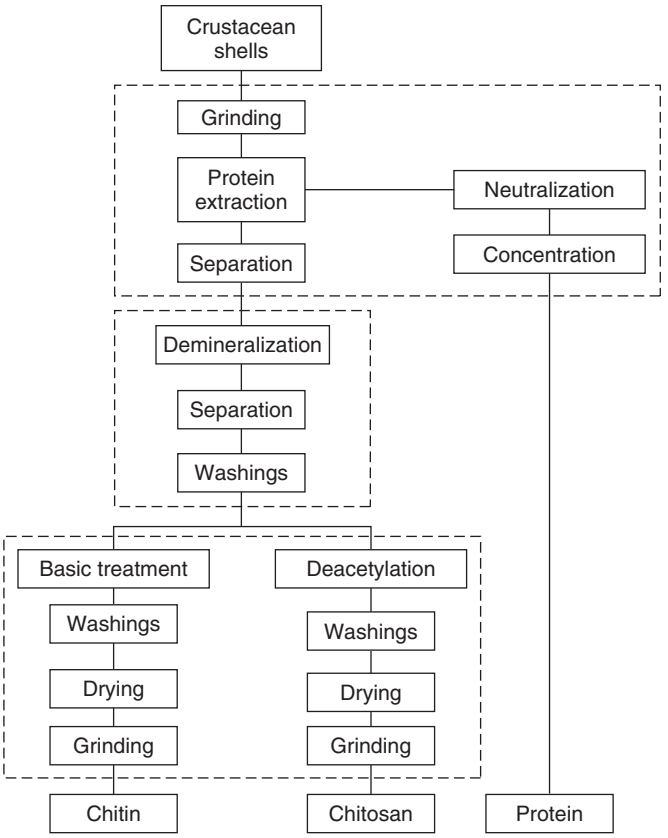

Figure 25.2 Schematic representation of the different steps involved in chitin/chitosan preparation procedures. In this diagram a second treatment with dilute NaOH is considered for chitin isolation in order to remove any residual protein.

(30–40 per cent), inorganic salts (mainly calcium carbonate and phosphate) (30–50 per cent) and lipids (0–14 per cent). These percentages vary considerably with the species and the season [8]. Consequently, the extraction techniques reported are very wide-ranging, since they depend significantly on the composition of the source. The majority of the techniques developed rely on chemical processes of hydrolysis of the protein and the removal of the inorganic material. A feasible sequence of isolation steps is schematically represented in Fig. 25.2. Some processes include a decolouration step of chitin by solvent extraction or oxidation of the remaining pigments. These isolation methods generally consume great amounts of water and energy, and frequently give rise to corrosive wastes. At present, enzymatic treatments are being investigated as a promising alternative. To this end processes have been reported that use enzymatic extracts or isolated enzymes and biological fermentations, but they still lack the effectiveness of the chemical methods, mainly with respect to the removal of the inorganic materials [9].

Chitin isolation processes are generally performed through the following consecutive steps: raw material conditioning, protein extraction (deproteinization), removal of inorganic components (demineralization) and decolouration. This sequence is preferred if the isolated protein is to be used as food additive for livestock feeding. Otherwise, demineralization can be carried out first [10]. A brief account of these processes will be given below. A more detailed description of chitin isolation (and chitosan preparation) can be found elsewhere [5, 8, 11].

25.2.1.1 Raw material conditioning

The crustacean shells are ground to the appropriate size (generally a few millimetres) and washed profusely with water to remove any organic material adhered to their surface. Acosta *et al.* have employed a pretreatment of the wastes with boiling water for 24 h followed by drying at 80°C for 24 h [12].

25.2.1.2 Deproteinization

The procedure most frequently used to separate the proteins consists in treating the crustacean shells with diluted aqueous NaOH solutions (1–10 per cent) at elevated temperatures (65–100°C). The reaction time usually varies from 0.5 to 72 h, depending on the treatment. Prolonged treatments or too high temperatures may provoke chain scission and partial deacetylation of the polymer. Sometimes, it is preferred to perform two consecutive treatments of 1–2 h. Other reagents employed for the removal of the proteins include: Na_2CO_3, $NaHCO_3$, KOH, K_2CO_3, $Ca(OH)_2$, Na_2SO_3, $NaHSO_3$, Na_3PO_4 and Na_2S [5].

The protein extracted can be recovered by lowering the pH of the solution to its isoelectric point for precipitation. The recovered protein can be used as a high grade additive for livestock starter feeds. This decreases the manufacturing costs of chitin.

The deproteinization processes using enzymatic extracts or isolated enzymes and biological fermentations have been tested with relative success, since they minimize the chemical degradation of chitin and lead to environmentally cleaner operations. However, enzymatic/microbiological treatments have the drawback of being time consuming and leaving the material with 1–7 per cent of residual protein [13]. These remnants can be diminished with the use of detergents [13] or with the application of physicomechanical techniques [14].

25.2.1.3 Demineralization

The main inorganic component of crustacean shells is calcium carbonate, which is usually eliminated with dilute HCl solutions (up to 10 wt-per cent) at room temperature, although other acids (*e.g.* HNO_3, HCOOH, HNO_3, H_2SO_4 and CH_3COOH) have also been employed. Demineralization occurs according to the following reaction:

$$CaCO_3 + 2HCl \rightarrow CO_2^{\uparrow} + CaCl_2 + H_2O \tag{25.1}$$

It is evident that if the amount of acid employed is below the stoichiometric ratio, the demineralization reaction will not be completed. The acid concentration and the reaction time depend on the source, but these parameters must be carefully controlled in order to minimize the hydrolytic depolymerization and deacetylation of chitin. High temperature treatments must also be avoided to prevent thermal degradation [10]. An alternative treatment for demineralization makes use of the complexing agent EDTA (ethylenediamine tetra acetic acid) in basic media [15].

25.2.1.4 Decolouration

The colour of crustacean shells is mainly due to the presence of pigments such as astaxanthin, cantaxanthin, astacene, lutein and β-carotene. The above treatments are not usually capable of eliminating these pigments which are frequently extracted at room temperature with acetone, chloroform, ether, ethanol, ethyl acetate or a mixture of solvents [8]. Traditional oxidizing agents such as H_2O_2 (0.5–3 per cent) and NaClO (0.32 per cent) have also been employed, but these reagents may attack the free amino groups and introduce modifications in the polymer. For strongly coloured shells, such as those of the common lobster carapaces, treatments with mixtures of acetone and NaOCl at room temperature have been reported [12].

25.2.2 Preparation of chitosan

Chitin deacetylation is performed by the hydrolysis of the acetamide groups at high temperature in a strongly alkaline medium. The reaction is generally carried out heterogeneously using concentrated (40–50 per cent) NaOH or KOH solutions at temperatures above 100°C, preferably in an inert atmosphere or in the presence of reducing agents, such as $NaBH_4$ or thiophenol, in order to avoid depolymerization. The specific reaction conditions depend on several factors, such as the starting material, the previous treatment and the desired degree of acetylation. Nevertheless, with only one alkaline treatment, the maximum deacetylation degree attained will not surpass 75–85 per cent. Prolonged treatments cause the degradation of the polymer without resulting in an appreciable increase in the deacetylation degree [16].

Several treatments have been developed to prepare fully deacetylated chitosan [17–19]. Their common characteristic is that they involve the repetition of consecutive deacetylation–washing–drying treatments as many times as required. Lamarque *et al.* developed a multistep heterogeneous deacetylation process for the production of well-defined chitosans with high deacetylation degrees and higher molecular weights than those usually reported

in the literature. The procedure was applied to α- and β-chitins in the presence of 50 per cent (w/v) NaOH, at temperatures ranging from 80°C to 110°C under an argon atmosphere [20].

Chitin, just like cellulose, is a semi-crystalline polymer, so that when deacetylation is performed in heterogeneous conditions, the reaction takes place mainly at the amorphous regions, whereas homogeneous conditions bring about a more uniform modification of the polymer. The latter reaction is carried out using alkali chitin, which is obtained by performing successive freezing–thawing cycles of an alkaline aqueous suspension of chitin until dissolution. Homogeneous deacetylation is achieved with more moderate alkali concentrations (about 13 wt-per cent), at 25–40°C for 12–24 h [21]. It has been shown that while chitosans obtained by the heterogenous process are polydispersed in terms of the acetylation degree of their chains, chitosans obtained under homogeneous conditions do not exhibit chain compositional dispersion.

The main drawback of the methods usually employed to produce chitosan is that they involve long processing times and expend large amounts of alkali. In order to overcome this, a number of unconventional deacetylation methods, such as thermomechanical processes using a cascade reactor operating under alkali conditions [22], flash treatment under saturated steam [23], microwave dielectric heating [14] and intermittent water washings [17], have been developed.

25.3 CHARACTERIZATION OF CHITIN AND CHITOSAN

25.3.1 Determination of the degree of acetylation

The degree of acetylation (or deacetylation) and the molecular mass are undoubtedly the most important parameters to establish the chemical and physical identity of chitin and chitosan. Both parameters vary with the biological source of the raw material and the preparation method. They also dictate the physicochemical, functional and biological properties of these polysaccharides, essential to fit an application or end product. Solubility, pK_o, viscosity, gelling capacity, among other properties, are all dependent on these parameters.

The degree of acetylation is defined as the fraction or percentage of *N*-acetylated glycosidic units in chitin or chitosan. It is designated indistinctly as F_A or DA (although DA is sometimes also expressed as DA per cent). It is also customary to express this parameter as degree of deacetylation ($= 1 - F_A$ or DD $= 100 -$ DA per cent). Table 25.1 shows a summary of the main spectroscopic, titration, circular dichroism, enzymatic, chromatographic, and thermal analytical methods that have been documented for the determination of DA in chitin and chitosan samples. Although the list of methods and references for each is not exhaustive, it purports to present an overview of the state-of-the-art of the various techniques, so as to serve as a guideline to help choose the best method to suit specific needs. To a great extent, the preferred method is influenced by the instrumental availability and the solubility of the sample. We have therefore separated the various analytical and instrumental methods in two categories, namely, those suitable for the analysis of samples in solution and those suitable for samples in the solid state. The former are best suited for the analysis of chitosan and the latter for both chitin and chitosan, regardless of their solubility. For routine analytical purposes of soluble chitosan, the measurement of *N*-acetyl content by the first-derivative UV-method gives very good results [24, 25], while IR spectroscopy remains the most simple and reliable method for samples of chitin or chitosan in the solid state [26–28].

It is important to recall that in addition to the net degree of acetylation, the distribution of the acetyl groups in the chitosan chain varies with the preparation protocol. Homogeneously deacetylated chitosan samples, with F_A varying in the range 0.04–0.49, showed that the ^{1}H-NMR diad frequencies distribution was close to random (Bernoullian). Conversely, chitosan samples within the same range of F_A, but produced under heterogeneous conditions, seemed to have a slightly more blockwise distribution [29]. This seems to be dependent on the degree of acetylation, as it is known that the alkaline reaction proceeds preferentially at the amorphous regions, and for chitosans with a $F_A > 0.44$, it will also proceed in the crystalline regions to give random type copolymers [30, 31]. This will result therefore, in the existence of a polydispersity with respect to DA among different chains on a chitin or chitosan sample. An important conclusion which also arises from these results is that the value of DA measured in a bulk heterogeneously prepared sample has an average character.

25.3.2 Molecular mass determination

The molecular weight and its distribution affect the physical, chemical and biological properties of chitosan, such as (i) the mechanical properties of hydrogels, (ii) the pore size of membranes, scaffolds and microcapsules [32],

Table 25.1

Characterization methods for determination of the degree of acetylation in chitin or chitosan

Method	Analytical conditions	Notes	References
I. Soluble samples			
High field ^{1}H NMR spectroscopy	Depolymerized chitosan samples dissolved in D_2O (pD 3–4) at 90°C	In all cases, DA is calculated from the relative areas of H-1 resonances of A and/or D or in combination with	[29]
	Native chitosan samples in DCl 2% at 70°C; pulse sequence: $\tau = 40$ s, $\theta = 45°$	*N*-acetyl group resonances to the total area of resonances	[210]
	Native chitosan samples in DCl 2% at 27°C; pulse sequence: $\tau = 13$ s, $\theta = 90°$	Acquisition time optimized based on inversion-recovery T_1 measurements	[211]
UV spectroscopy	Chitosan dissolved in 0.01 M acetic acid; calibration curve with of *N*-acetyl glucosamine	First derivative of UV absorbance is recorded at the point of null contribution of acetic acid ($\lambda \sim 200$ nm)	[24, 25]
Circular dichroism	Chitosan dissolved in acetic acid; calibration curve with *N*-acetyl glucosamine	Based on n–π^* transition of amide groups with a c.d. band located in the region of $\lambda \sim 211$ nm	[212]
Potentiometry	Chitosan dissolved in excess of HCl	Titration with NaOH measures the consumption of HCl needed to protonate the amino groups	[11, 213]
Conductimetry	Chitosan dissolved in excess of HCl	Titration with NaOH needed to neutralize the free amino groups in chitosan	[214]
Enzymatic method	Chitosan dissolved in 0.2% acetic acid and incubated with 5 units of each exo-β-D-glucoseaminidase, β-*N*-acetylhexosaminidase and chitosanase for 12 h at 40°C	Liberated amounts of glucosamine and *N*-acetyl glucosamine monosaccharides are determined by a specific colorimetric or HPLC method	[215]
II. Insoluble samples			
FTIR spectroscopy	Ground samples are mixed with dry KBr (~1:100) (*NB*: thin films can also be cast from concentrated solutions of soluble samples)	Several sets of measuring and reference bands calibrated with DA determined by NMR and UV spectroscopy and titration methods	[26–28]
^{13}C CP-MAS NMR	No sample preparation	Relaxation delay optimized. DA is calculated from the relative areas of CH_3 to the total resonances	[214, 216]
^{15}N CP-MAS NMR	No sample preparation	Not suitable for DA < 10%. Particularly useful for chitin/chitosan complexes associated with other polysaccharides	[217, 218]
Elemental analysis	Powder analysed directly	Calculated from the C/N ratio	[219]
Thermal analysis	Powder analysed directly	Based on the enthalpy of degradation of sample	[220]
Pyrolysis – GC	Pyrolysis at 450°C under He	DA calculated from programs of sample and of *N*-acetylglucosamine	[221]
HPLC	Sample hydrolyzed with 2.41 M H_2SO_4	Based on the determination of liberated acetyl groups	[222]

(iii) the particle size and drug release properties of nanoparticles [33], (iv) the effect of chitosan on the permeability of epithelial cells [34] and (v) its antimicrobial activity [35]. Therefore, this parameter affects directly the performance of chitosan in biotechnology, food, pharmaceutical, biomedical and biological applications.

The main methods used to determine the molecular weight of chitin and chitosan are similar to those used for any polymer, namely, viscosimetry, light scattering, gel permeation chromatography, osmometry and sedimentation equilibrium by ultracentrifugation. The first three are briefly discussed in the following subsections since they are the most employed techniques.

25.3.2.1 Viscosimetry

Viscosimetry is the mostly utilized method to determine the molecular weight of chitosan due to its simplicity. The method has the disadvantage of not being absolute because it relies on the correlation between the values of intrinsic viscosity with those of molecular weight, as determined by an absolute method for fractions of a given polymer. This relationship is given by the well-known Mark–Houwink–Sakurada equation.

$$[\eta] = KM_{\upsilon}^{a} \tag{25.2}$$

where M_{υ} is the viscosimetric-average molecular weight and K and a are constants that depend on the nature of the polymer, the solvent system and the temperature. Experimentally, the intrinsic viscosity of chitosan solutions is calculated using the Huggins and Kraemer equations [36].

The degree of acetylation and the polydispersion with respect to the size and the content of residual *N*-acetylated groups must be known for the calibration step, given their influence on the rheological behaviour of the corresponding solutions. The tendency of chitosan to form aggregates must also be taken into account. An exhaustive updated list of calibration constants obtained under different conditions and solvent systems has recently been reviewed [37]. The constants given by Rinaudo [38] are perhaps, to date, the most realistic ones, as they call upon a calibration from size exclusion chromatography and hence a large number of monodisperse fractions. This issue is further discussed in Section 25.3.4.

25.3.2.2 Static light scattering

Static light scattering (SLS) provides the direct determination of the polymer weight-average molecular weight, M_{w}, along with the *z*-average radius of gyration, $<S^2>^{1/2}$, and the second virial coefficient, Γ_2, without need of calibration [39]. The angular and concentration dependence of the excess Rayleigh ratio $R_{\mathrm{vv}}(q)$ of dilute polymer solutions is measured and related to M_{w}, $<S^2>^{1/2}$ and Γ_2 by the following equation:

$$\frac{KC}{R_{\mathrm{vv}}(q)} = \frac{1}{M_{\mathrm{w}}}\left(1 + \frac{1}{3}\left\langle S^2\right\rangle^{1/2} q^2\right) + 2\Gamma_2 C \qquad C \to 0;\ \theta \to 0 \tag{25.3}$$

where, for vertically polarized incident radiation, the optical constant is given by $K = 4\pi^2 n_{\mathrm{o}}^2 (dn/dC)^2/(N_{\mathrm{A}}\lambda_{\mathrm{o}}^4)$ and $q = (4\pi n_{\mathrm{o}}/\lambda_{\mathrm{o}})\sin(\theta/2)$, with N_{A}, n, C, λ_{o} and θ being Avogadro's constant, the solvent refractive index, the polymer concentration ($\mathrm{g\,cm^{-3}}$ or $\mathrm{gg^{-1}}$), the light wavelength in the vacuum, and the scattering angle, respectively. In order to calculate M_{w}, $<S^2>^{1/2}$ and Γ_2 using Eq. (25.3), the refractive index increment, *dn/dC* for a given polymer–solvent system, must also be determined independently (*i.e.* using an interferometer) with high precision. The *dn/dC* values of chitosan in 0.15 M ammonium acetate/0.2 M acetic acid buffer (pH 4.5) have been found to vary from 0.195 to 0.154 for chitosans with DA values of 5.2 and 70.6, respectively [40]. The simultaneous double extrapolation to both zero angle and zero concentration is conducted by constructing the well-known Zimm plot.

Despite being an absolute method that provides very important information on the fundamental properties of the polymer in solution, including its conformation, chain stiffness and its interaction with the environment, the use of SLS is limited as it requires expensive equipment and strict experimental conditions including very pure and fully soluble polymers and dust-free. In addition, Zimm plots of experimental data can be irregularly shaped and difficult to interpret when polymer aggregation or association occurs [41]. Several examples of further studies addressing the macromolecular dimensions and conformation of chitin [42] and chitosan [40, 43–48] can be found in the literature.

25.3.2.3 High performance size exclusion liquid chromatography

Size exclusion chromatography coupled with HPLC (SEC–HPLC or HPSEC) permits not only the determination of the molecular weight, but also its statistical distribution, hence the index of polydispersity [49]. Separation of the polymer species takes place according to their molecular size, or more strictly, their hydrodynamic volume. This method is claimed to be rapid, reproducible and simple compared with light scattering, osmometry or ultracentrifugation.

By coupling a refractive index detector to the high performance size exclusion liquid chromatography (HPSEC) system, it is possible to determine the amount of polymer eluting at a given retention time (t_R) or retention volume (V_R). In order to determine the molecular weight using such an instrumental set up, the system must be calibrated in terms of the expected V_R of a polymer fraction of known M_w. Since there are no chitosan standards, in order to construct a universal calibration curve, pullulan standards of narrow M_w distribution have been used, as for other polysaccharides. Isogai has reviewed the various methods for obtaining the molecular mass of chitin and chitosan by HPSEC, with emphasis on methodological aspects and solvent systems [50]. An ASTM guide describes the application of this technique to chitosan [51].

It is crucial that the separation mechanisms in HPSEC are determined by the size of the molecules and not by their charge, nor by ion exclusion effects or adsorption into the column. These remarks are particularly relevant to chitosan which is a polyelectrolyte. The addition of sodium acetate or ammonium acetate in the buffer used as the mobile phase generates enough ionic strength to overcome these unwanted features.

In order to avoid the use of standards, multidetection SEC–HPLC systems incorporate, in addition to the refractive index detector, a multiple angle laser light scattering (MALLS) detector. This enables the determination of the molecular weight and radius of gyration of the individual fractions as they elute out of the column, hence to obtain their distribution as a function of concentration. Multidetection HPSEC is undoubtedly the preferred technique for the analysis of the distribution of chitosan's molar masses and sizes [38, 52, 53].

The MALLS detector uses the same fundamental principles of light scattering discussed above (Eq. 25.3). Other detectors that can be coupled to a multidetection system include viscosity and dynamic light scattering units, which enable the determination of additional fundamental parameters providing information on the molecular shape and conformation of chitosan in solution. Thus, using the viscosity detector coupled to the MALLS counterpart, Brugnerotto *et al.* derived the Mark–Houwink–Sakurada K and a constants (Eq. 25.2) for chitosans of varying DA [54].

25.3.3 Crystallinity

Chitin is found in nature as highly crystalline ordered networks. Two major polymorphs have been firmly established for chitin in nature, namely α and β-chitin. Each can readily be distinguished on the basis of its X-ray diffraction patterns [55], solid state ^{13}C CP-MAS NMR [56], infrared [57] and Raman spectroscopy [58].

α-Chitin is the most stable and ubiquitous form of chitin occurring in the exoskeleton of arthropods and in fungi. Its chains are arranged along a two-chain orthorhombic unit cell with dimensions $a = 0.474$, $b = 1.032$ and $c = 1.886$ nm [55]. Space groups are consistent with a P_{212121} symmetry, which requires the antiparallel arrangement of the chains.

On the other hand, β-chitin occurs commonly in squid pen [59], in diatoms and in deep-sea organisms (*e.g. Tevnia jerichonana*) [60] from which it has been isolated. The early crystallographic studies established that the unit cell of β-chitin is monoclinic and has a P_{21} space group, with its chains arranged in a parallel structure. In contrast with α-chitin, β-chitin can incorporate small molecules into its crystal lattice to form various complexes, in particular anhydrous, monohydrated and dehydrated forms have been identified [61]. The inclusion of water between the dense sheet planes formed by the close stacking of chains leads to an increase in the distance between sheets along the intersheet axis (c axis). Recent X-ray crystallographic studies carried out with highly crystalline β-chitin have provided intersheet (c axis) d-spacing values of 0.92, 1.03 and 1.1 nm for anhydrous, mono- and dehydrated forms, respectively [62]. Differential scanning calorimetry (DSC) evidence showed that the incorporation of water in highly crystalline β-chitin is reversible and that the thermal transitions between the various forms can be induced by heating and cooling cycles. Yet another key feature of β-chitin is its ability to undergo conversion into its α-chitin polymorph by precipitation from a formic acid solution or by treatment with cold 6 M HCl [63].

Highly deacetylated chitosan in the solid state can also exhibit a crystalline structure. Two hydrated crystalline forms of chitosan have been identified, namely 'L-2' and 'Tendon', the latter being the most abundant hydrated polymorph of chitosan [64].

25.3.4 Solution properties

Because of its high cohesive energy related to strong intermolecular interactions through hydrogen bonds, chitin like cellulose is difficult to dissolve. Roberts has grouped the solvent systems for chitin into three categories *viz.* aqueous solutions of neutral salts, acid solvents and organic solvents [5]. The former group rarely gives good solutions, while the solvents of the second class generally provoke degradation of the polymer chain. Up to recent years, the most common solvent for chitin was the system composed by *N*,*N*-dimethyl acetamide (DMA) containing LiCl in amounts up to 8 per cent. It has been recently shown that chitin can be dissolved in calcium chloride dihydrate–saturated methanol and that this system is stable for long periods of time at room temperature [65].

Vincendon showed that chitin dissolves in DMA–5 per cent LiCl via strong interaction of one LiCl molecule with intermolecularly hydrogen bonded labile protons (OH or NH) of *N*-acetylglucosamine residues [66]. This interaction is assumed to destroy the intermolecular hydrogen bonds, allowing chitin to swell and then to dissolve. Terbojevich *et al.* have demonstrated that chitin chains are rather stiff in DMA–5 per cent LiCl and have a high persistence length, ranging from 150 to 400 Å [67]. A later paper by this group estimated the Mark–Houwink constants as $a = 0.88$ and $K = 0.021\,\mathrm{mLg^{-1}}$ in this solvent system at 25°C [42].

In contrast to chitin, the presence of free amino groups along the chitosan chains allows this macromolecule to dissolve in dilute aqueous acidic solvents through the protonation of these groups and the formation of the corresponding chitosan salt. It is therefore important to realize that the polyelectrolyte character of chitosan influences its solution properties.

25.3.4.1 Acid-base properties

An important parameter that characterizes the polyelectrolyte behaviour of this weak polybase is its intrinsic pK, pK_0, which is the pK-value extrapolated to zero charge. According to Katchalsky, the apparent value of pK, pKa, is defined by [68]

$$\mathrm{pKa} = \mathrm{pH} + \log\frac{1-\alpha}{\alpha} = \mathrm{pK}_0 - \frac{\varepsilon\Delta\Psi(1-\alpha)}{kT} \tag{25.4}$$

where Ka is the dissociation constant of the conjugate acid *viz.*

$$\sim\!\!\sim\!\!\sim \mathrm{NH_3^+} \underset{}{\overset{\mathrm{Ka}}{\rightleftharpoons}} \sim\!\!\sim\!\!\sim \mathrm{NH_2} + \mathrm{H^+} \tag{I}$$

A study on this issue has shown that Ka depends largely on the degree of neutralization, particularly at low degrees of *N*-acetylation [69]. For a given value of α, the higher the acetyl content, the higher the pKa value. The computed value for pK_0 (6.5) was shown to be independent of the degree of acetylation of the sample up to 0.25. Nevertheless, investigations carried out by Rinaudo *et al.* for chitosan hydrochloride and acetate, combining potentiometric, conductimetric and viscosimetric experiments, gave a pK_0 value of 6.0 ± 0.1 [70, 71], that is, lower than that reported for D-glucosamine (between 7.5 and 7.8), indicating the influence of the chemical environment on the strength of this polybase.

25.3.4.2 Conductimetric behaviour

According to Manning's theory for polyelectrolytes [72], a charged macromolecule in solution is submitted to *counterion condensation* when the so-called charge parameter, ξ, is higher than unity, enabling this parameter to decrease.

Since the charge parameter is proportional to the charge density, the former depends on the degree of *N*-acetylation and the degree of dissociation of the amino groups and therefore on the solution pH. For chitosan, the charge parameter becomes [38]

$$\xi = 1.38 \times (1 - \mathrm{DA}) \times \alpha \tag{25.5}$$

where DA is the degree of *N*-acetylation and α the degree of protonation. The expression

$$\chi = cf(\lambda_P + \lambda_c) \tag{25.6}$$

defines how the specific conductivity, χ, is affected by the presence of the polyelectrolyte following Manning's theory [72]. Here, c is the polyelectrolyte concentration, λ_P and λ_c are the limiting equivalent conductivities for the polyelectrolyte and the corresponding counterion, respectively, and f is the transport coefficient which is directly related to the charge parameter.

The values for a fully protonated chitosan chain with DA = 0.20 in the presence of univalent counterions has been estimated as $f = 0.82$ and $\lambda_P = 31.4 \times 10^{-4}\,m^2 \cdot S\ mol^{-1}$ [73], the former being in good agreement with the theoretical value ($f = 0.79$).

25.3.4.3 Dilute solution properties

The most relevant information about the behaviour, size and conformation of macromolecules must come from studies of dilute solutions. Many such investigations have been published and controversies have arisen which have not yet been solved.

Concerning the chemical composition, two factors must be taken into account when examining the stiffness of chitosan chains, namely (i) the presence of acetylated units should increase the rigidity because of steric reasons and/or hydrogen bonding between two adjacent residues and (ii) the polyelectrolyte character of this biopolymer derived from the presence in solution of ammonium ions will tend to expand the chains at low or moderate ionic strength, thus increasing the excluded volume by electrostatic repulsions. It is apparent that each of these two factors will increase when the other decreases, sometimes counterbalancing each other depending on their relative strength.

The analysis of the stiffness parameter, ***B***, proposed by Smidsrød and Haug [74] for samples of chitosan with different degrees of acetylation led to an important conclusion. At contents of amino groups of about 80 per cent there is no effect of chemical composition on the chain stiffness [38, 75–77]. However, when the content of *N*-acetylglucosamine residues increases, the rigidity of the polysaccharide increases indicating that the role of acetyl groups dominates over the polyelectrolyte effect. (It should be remembered that the higher the stiffness of the polyelectrolyte chain, the lower the ***B*** value). A possible explanation for this behaviour is that if the charge parameter of a fully protonated chitosan chain is evaluated (see Eq. 25.6), the result is that for DA ≥ 0.28, $\xi \geq 1$. On the contrary, when DA < 0.28, then $\xi < 1$, making the charge density lower and the role of acetyl groups more important than the polyelectrolyte effect.

The worm-like chain has proved to be an appropriate model for studying the conformational characteristics of chitosan. Considering that the persistence length is a parameter that characterizes the stiffness of a worm-like chain, many authors have evaluated it with conflicting results [38, 76, 78], denoting that many factors influence its experimental determination.

A study carried out with chitosan samples prepared by both homogenous and heterogeneous processes helps to understand the role of the *N*-acetyl group content and its distribution on the stiffness of the polymer chains. Theoretical and experimental data have shown that the intrinsic persistence length remains constant ($L_P = 110$ Å) for heterogeneous chitosans with degrees of acetylation lower than 25 per cent. For homogeneous chitosans, L_P increases with the degree of acetylation. The stiffness of chitosan chains appears greater for homogeneously *N*-reacetylated chitosan [79].

25.3.4.4 Rheological behaviour

The investigation of the rheological behaviour of chitosan solutions has been carried out by studying the influence of different parameters such as chemical composition, concentration, ionic strength, pH and the effect of the type of acid [54, 80–85]. Furthermore, the role of hydrophobic association phenomena on the viscoelastic behaviour of chitosan solutions is an interesting feature [86] which requires further understanding.

25.4 INTERACTION WITH METAL IONS

The capacity of chitin and chitosan to form complexes with metal ions, particularly with transition and post-transition metal ions, has been widely documented and comprehensive reviews on the subject are available [11, 87, 88].

However, in spite of this vast literature it is generally difficult to compare the results from different authors. This is because the adsorption capacity and the rate of adsorption of metal ions by these biopolymers depend on a number of factors such as the physical state of the polymer (powder, flakes, or even if was reprecipitated), its degree of crystallinity, the degree of acetylation and the chain length. Temperature, stirring rate, contact time with the metal ion solution, pH and ionic strength, are also factors that influence adsorption.

Various authors have reported different ordering for the metal ions sorption capacity of chitosan. For instance, while Muzzarelli and Tubertini conclude that the adsorption capacity of chitosan follows the Irvin and Williams series [89], Koshijima *et al*. [90] order it in the series Cr(III) < Co(II) < Pb(II) < Mn(II) < Cd(II) < Ag(I) < Ni (II)< Fe(II) < Cu(II) < Hg(II) and Masri *et al.* [91] arrange them as follows: Cr(III) < Fe(II) < Mn(II) < Co(II) < Cd(II) < Cu(II) < Ni(II) < Ag(I) < Pb(II) < Hg(II).

Chitosan exhibits a superior metal ion sequestering ability than chitin. The presence of the amino groups in the structural unit of chitosan is definitely the main cause of its complexing capacity, but the binding mechanism of metal ions to chitosan is not yet completely understood. Various processes such as adsorption, ion exchange and chelation have been considered as the mechanisms responsible for complex formation between chitosan and metal ions. The type of the interaction prevailing depends on the metal ion, its chemistry and the pH [92]. Ogawa *et al.* studied the complex formation of chitosan with transition metal ions [93] by immersing stretched films of chitosan (DD = 99.5 per cent) in 0.1–0.4 M solutions of Co(II), Ni(II), Cu(II), Zn(II) and Hg(II) salts for 1 h. The films were then washed, dried and inspected by X-ray diffraction. The first structure they proposed as the most probable for these complexes involved the binding of the pendant metal ion to one amino group of the dimer residue of chitosan.

The generally accepted interpretation at present is that under heterogeneous conditions at pH < 6, chitosan acts as a poly(monodentate) ligand, while at higher pH, it behaves as a poly(bidentate) ligand, forming chelates. However, in solution, the formation of complexes in which two amino groups (belonging to the same chain or to two different chains) are coordinated with the same metal ion, can also be envisaged [5].

The elevated metal ions adsorption capacity of chitosan is of course particularly interesting for applications in different contexts such as metal ion recovery from solutions, decontamination of residual industrial waters, inorganic chromatography or catalyst support. To this end, a number of chitin and chitosan derivatives with improved properties have been prepared. Thus, chitosan has been crosslinked with di/polyfunctional reagents, such as glutaraldehyde for preventing its dissolution in an acid medium. Other modifications include the synthesis of polyampholytes with increased chelating ability (*N*-carboxymethyl and *N*-(*O*-carboxybenzyl) chitosan [94]); derivatives containing sulphur, such as chitosan dithiocarbamate [95] and *N*-(2-hydroxy-3-mercaptopropyl) chitosan [96], with superior sequestring capacity for Hg^{2+}, and grafting with other polymers, like polyacrylonitrile [97] and polyvinylpyrrolidone [98]. The structure of some of these chitosan derivatives are shown in Fig. 25.3. A more comprehensive account of chitin and chitosan modifications for ion binding purposes can be found elsewhere [88].

25.5 CHITOSAN IN POLYELECTROLYTE COMPLEXES

Polymers exhibit a high tendency to interact and generate interpolymer complexes and hence supramolecular structures. These interactions constitute the basis of many biological reactions occurring in living organisms. They also provide a route to the preparation of novel polymeric materials displaying physical properties different from those of the individual constituent macromolecules. As a result, in the last few years many studies have been devoted to the elucidation of the nature of these interactions as well as to the evaluation of the physical properties of the resulting materials [99–103]. Interpolymer complexes are often classified according to the nature of their interactions such as charge-transfer or coordination complexes, stereocomplexes resulting from van der Waals forces, hydrogen-bonded complexes and polyelectrolyte complexes (PEC) [99].

As a cationic biopolymer, chitosan may react with any other anionically charged polyelectrolyte, giving rise to the formation of PEC [101, 104]. There are reports of PEC between chitosan and carboxymethyl cellulose (CMC) [105–108], alginate [109–112], poly(acrylic acid) [107, 113, 114], pectin [73, 115–117], carrageenans [118–121], heparin [122] and others [123–131].

Gene therapy is currently one of the most advanced strategies to solve important health problems such as cancer, AIDS and cardiovascular diseases. The ideal gene delivery system must be capable of protecting the DNA during the transfer of genetic material until it reaches the target cells of a patient. Chitosan is a good candidate for gene delivery systems through the formation of PEC with negatively charged DNA [132–134].

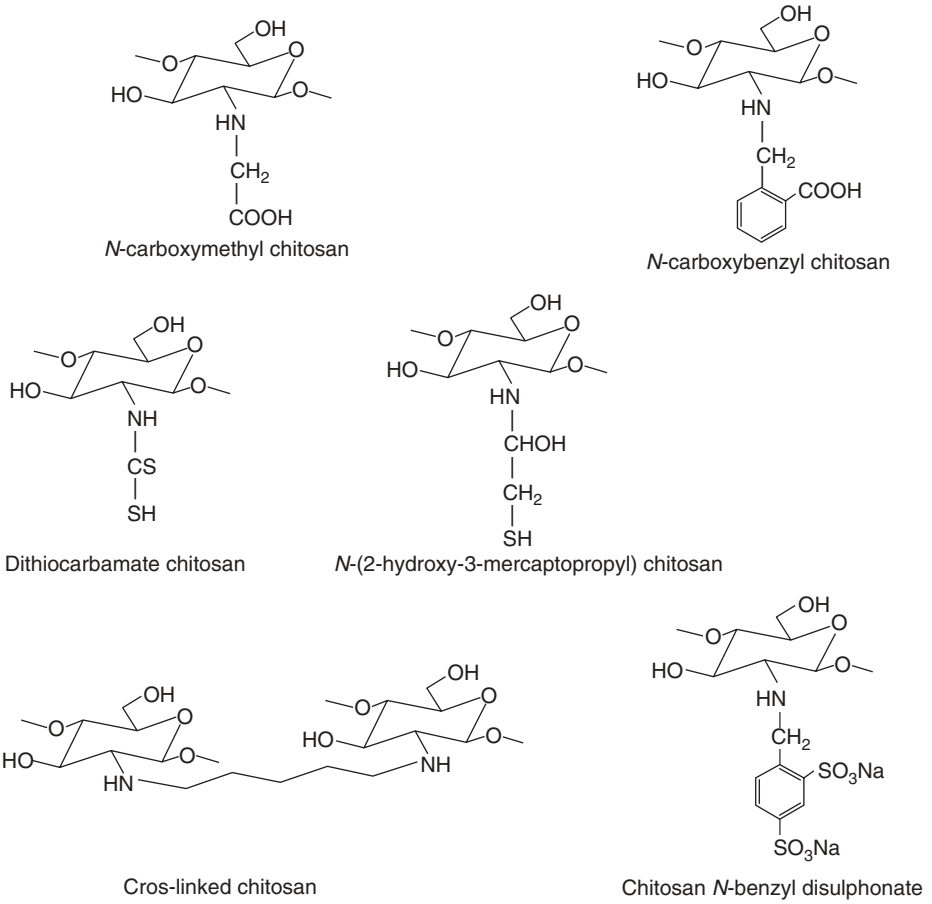

Figure 25.3 Some of the many chitosan derivatives developed for increasing its performance as metal ion scavenger.

The interaction of chitosan with carboxylic polyacids leads to the formation of an insoluble PEC. Chitosan is insoluble in neutral and basic media, therefore the stoichiometric PEC is usually obtained by mixing equimolar quantities of the polyacid and chitosan hydrochloride. The complex is formed according to the following reaction:

$$\underset{C_0(1-\theta)}{\sim\!\sim\!\sim COOH} + \underset{C_0(1-\theta)}{{}^{+}NH_3\!-\!\sim\!\sim} \rightleftharpoons \underset{C_0\theta}{\sim\!\sim\!\sim COO^-} \; {}^{+}NH_3\sim\!\sim\!\sim + \underset{C_0\theta}{H^+} \qquad \text{(II)}$$

As a result, the pH of the solution decreases. The degree of conversion of the complex, θ, expressed as the ratio of the concentration of interchain salt bonds formed, C_k, to the initial concentration of any of the polyelectrolytes, C_0, can be evaluated from potentiometric measurements by:

$$\theta = \frac{([H^+] - [H^+]_{PA})}{C_0} \qquad (25.7)$$

where $[H^+]$ and $[H^+]_{PA}$ are the concentration of hydrogen ions in the reaction mixture and in a solution of the polyacid at the same concentration, respectively. However, the equilibrium (II) can be shifted to the right by addition of a strong base. In so doing, θ can be assessed from the data of the potentiometric titration of the mixture.

Reactions between polyelectrolytes are accompanied by the release into the medium of ionic species with different mobilities, thus making conductimetry a powerful technique to study these types of processes. Taking into

account the additivity of the contributions of all ionic species, the knowledge of the conductivity of the reaction medium allows the degree of conversion to be determined [73, 111].

The stoichiometry of PECs of weak polyacids and weak polybases is pH dependent, because of the variation of their dissociation degree with pH. The composition of the PEC is then given by:

$$\alpha_{PA}[PA] = \alpha_{PB}[PB]$$

where [PA] and [PB] are the molar concentrations of the polyacid and the polybase in the PEC and α_{PA} and α_{PB} their respective dissociation degrees [99, 111].

An important characteristic of interpolyelectrolyte reactions is their cooperativity, which is determined by the influence of neighbouring functional groups on the reactivity of the next one. The cooperativity of interpolyelectrolyte reactions is well-illustrated in the case of the PEC between chitosan and poly(acrylic acid) [135]. The equilibrium (II) can be decomposed into the following contributions:

$$\sim\!\!\sim COOH \underset{}{\overset{K_d}{\rightleftharpoons}} \sim\!\!\sim COO^- + H^+ \qquad \text{(III)}$$

$$\sim\!\!\sim COO^- + {}^+NH_3 \sim\!\!\sim \underset{}{\overset{K_2}{\rightleftharpoons}} \sim\!\!\sim COO^{-+}NH_3 \sim\!\!\sim \qquad \text{(IV)}$$

The existence of cooperativity implies that the equilibrium constant for the formation of bond i, K_2^i, is bigger than that for the formation of bond $i-1$, K_2^{i-1}. The magnitude of this effect can be adequately quantified by expressing K_2 as $K_2 = K_2^0 \times \exp^{-m\theta}$, where K_2^0 is the equilibrium constant for the formation of the first interchain bond [136]. A marked increase in K_2 with θ, denotes a cooperative reaction resulting in the formation of long sequences of consecutive bonds [135].

Most of applications of PEC are in membranes. The wall of microcapsules consists of a membrane of PEC, whose characteristics govern properties that are usually decisive for their end use. The properties of PEC membranes are strongly dependent on the preparation conditions, *viz.* pH, ionic strength, temperature, concentration and molar ratio of reacting polyelectrolytes, among others [137]. These membranes adsorb water until a maximum swelling is achieved after which they slowly shrink to an equilibrium size [110, 115, 137]. The contraction experienced by the PEC membranes can be explained considering two factors: (a) the small ions trapped diffuse out of the membrane, decreasing the osmotic pressure and (b) at pH 5.5 the free carboxylic groups of the CMC chains are mostly as sodium carboxylate and the amino groups of chitosan are protonated, so that new salt bonds can be formed. The segmental mobility in the swollen state must be sufficient for this reaction to proceed [137]. As a result, θ rises with time, increasing the retractile force of the polymer network.

However, if the membrane is dried after a first swelling cycle, further swelling sequences show the typical pattern of a polymer network. This behaviour indicates that the rearrangements of the polymer chains during the first cycle, which induces an increase in the degree of complexation, no longer take place [110].

During the swelling process, the integrity of the PEC network is maintained by the crosslinking of chains produced by the interchain —NH_3^+ $^-$OOC— salt bonds. However, at low or high pH, these bonds can be broken, resulting in the disintegration of the PEC and the dissolution of the membrane. This pH instability can be modified by heating PEC membranes at 120°C under a nitrogen atmosphere, a process which forms covalent amide bonds according to the following reaction [117, 138, 139]:

$$\left|\!\!-COO^{-+}H_3N-\right| \xrightarrow{\Delta T} \left|\!\!-COO-HN-\right| + H_2O \qquad \text{(V)}$$

The membrane then becomes insoluble whatever the solution pH and moreover, the degree of swelling at a given pH decreases as a result of reaction (V). The extent of amide bond formation for a given membrane depends on the heating time, and therefore this parameter can be used to regulate the swelling behaviour of these PEC membranes.

It is interesting to note that when chitosan/CMC PEC membranes in the swollen state are placed in solutions containing $CaCl_2$, a collapse in volume is observed [140, 141]. This effect is not induced by other salts, such as NaCl, KCl, $NaNO_3$ and Na_2SO_4, indicating the existence of a specific role of the Ca^{2+} ions on the free carboxylate groups of the PEC, possibly through the formation of Ca^{2+} complexes with fixed ligands of the network. This complexation

can be visualized as the formation of new crosslinks, which would increase the retractile force of the PEC's macromolecular network, thus provoking its contraction. When the pH of the formation of the membranes is increased, this effect is enhanced because of the greater amount of available free carboxylate groups. The variation of the swelling degree with pH and Ca^{2+} concentration can be exploited to control the permeability of solute fluxes through the membranes [140].

25.6 APPLICATIONS IN MEDICINE AND PHARMACY

Chitosan is a very useful polymer for biomedical applications because of its biocompatibility, biodegradability and low toxicity. Chitin has also been described as biocompatible and biodegradable, and has found applications for specific purposes, such as sponges and bandages for the treatment of wounds and suture threads. However, it has generally received less attention than chitosan, because of its insolubility in water and low reactivity.

The biodegradability of chitin and chitosan is mainly due to their susceptibility to enzymatic hydrolysis by lysozyme, a non-specific proteolytic enzyme present in all tissues of the human body. Lipase, an enzyme present in the saliva and in human gastric and pancreatic fluids, can also degrade chitosan [142]. The products of the enzymatic degradation of chitosan are non-toxic. The degree of acetylation, the molecular weight, the pH and even the method of preparation of chitosan affect biodegradation.

In contact with blood, chitosan activates the formation of clots as a result of the interaction of the amino groups with the acid groups of blood cells [143]. It is therefore a good haemostatic agent. However, it has been found that water-soluble chitosan and chitosan oligomers do not present thrombogenic activity, whereas the sulphated derivatives of chitosan exhibit anticoagulant activity [143]. It has also been claimed that chitosan is hypocholesterolemic and hypolipidemic [144]. It has antimicrobial, antiviral and antitumoural activity [145]. The immunoadjuvant activity of chitosan has also been recognized [146].

All these interesting characteristics have led to the development of numerous applications of chitosan and its derivatives not only in biomedicine such as surgical sutures, biodegradable sponges and bandages [143], matrices (in microspheres, microcapsules, membranes and compressed tablets) for the delivery of drugs [147], but also in orthopaedic materials and dentistry [148].

25.6.1 Chitosan as a biomaterial

The great variety of applications of chitosan in the field of biomaterials is due to its excellent properties when interacting with the human body: bioactivity, antimicrobial activity, immunostimulation, chemotactic action, enzymatic biodegradability, mucoadhesion and epithelial permeability which supports the adhesion and proliferation of different cell types [149].

Chitosan has been tested for applications such as contact lenses, tissue adhesive, preventing bacterial adhesion, sutures and others [150]. However, this biopolymer has been thoroughly investigated mainly in two biomedical fields. On the one hand, it has been used together with chitin in the treatment of wounds, ulcers and burns, calling upon its haemostatic properties and its accelerating wound healing effect. On the other hand, given its cell affinity and biodegradability, it has been applied in tissue regeneration and restoration, including its perspective use as a structural material in tissue engineering.

25.6.1.1 Treatment of wounds and burns

This is undoubtedly one of the most promising medical applications for chitin and chitosan. The adhesive properties of chitosan, together with its antifungal and bactericidal character, and its permeability to oxygen, are very important properties associated with the treatment of wounds and burns. Different derivatives of chitin and chitosan have been patented for this purpose in the form of membranes, woven fibres, hydrogels, etc. Some of these formulations have been released on the market, like *Beschitin* in Japan (based on chitin) or *HemCon* in USA (based on chitosan).

Both polysaccharides promote granulation (with angiogenesis) and cell organization during wound healing. The reepithelization and tissue regeneration are strongly improved in open wounds, and at the same time scarring is reduced. The analgesic effect of both of chitin and chitosan has also been reported [151].

PEC of chitosan and heparin have been also studied as matrices for wound healing, because it has been demonstrated that heparin interacts and stabilizes growth factors involved in the wound healing process [152].

25.6.1.2 Tissue engineering

Tissue engineering techniques generally require the use of three-dimensional (3D) supports for initial cell attachment and subsequent tissue formation. Chitosan has similar structural characteristics as glycosaminoglycans (GAGs) found in extracellular matrix of several human tissues. It has therefore been widely employed in tissue engineering, since it facilitates cell attachment and the maintenance of differentiating functions. Gelatin/chitosan or collagen/chitosan supports (some of them crosslinked with glutaraldehyde) have been investigated with promising results in cell growth and proliferation in tracheal [153], cartilage [154], nerve [155] and bone tissue [156] repair and regeneration. In the latter context, a composite with hydroxyapatite was used. Chitosan/chondroitin sulphate membranes have been shown to support chondrogenesis and are, therefore, promising materials for cartilage repair [157].

Platelet-derived growth factor releasing chitosan/chondroitin sulphate sponges have also been evaluated in bone regeneration [158]. A human skin equivalent composed of collagen–GAG–chitosan has been reported [159]. The biological properties of chitosan–chondroitin sulphate and chitosan–hyaluronate PEC have been studied [160]. These materials were cytocompatible, but better cell attachment and proliferation was attained with pure chitosan. Chitosan–GAG materials, including chitosan–heparin, have been evaluated as modulators of vascular cells proliferation. In this same vein, antigenic collagen–hyaluronic acid mixtures have been proposed to promote fibroblasts growth for tissue repairing.

Porous 3D scaffolds of chitosan/calcium phosphate composites have also been proposed for bone regeneration [148]. Calcium phosphate greatly reinforced the chitosan matrix and also modulated the release burst effect, when loaded with gentamicin. In addition, good cellular biocompatibility was observed. The preparation of chitosan/tricalcium phosphate sponges by mixing and freeze drying, in some cases loaded with platelet-derived growth factor, has also been reported [161].

An interesting strategy in tissue engineering is the encapsulation or immunoisolation of pancreatic and hepatic cells [162] (Fig. 25.4). Langerhans islets have been enclosed in chitosan/calcium alginate capsules with the aim of developing an artificial pancreas for the treatment of diabetes mellitus [163].

25.6.2 Pharmaceutical applications

Chitosan has been widely used as matrix in drug-release systems in the form of beads and granules, as promising vehicles for oral drug sustained-release formulations [164].

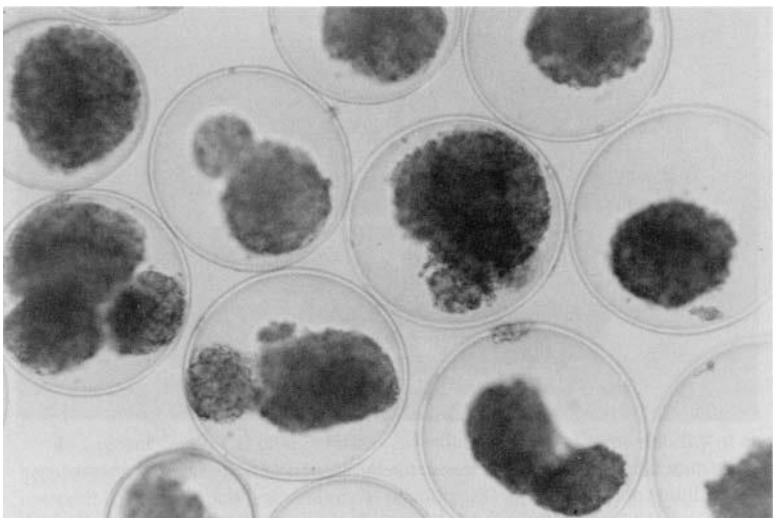

Figure 25.4 Cells enclosed in chitosan/calcium alginate microcapsules prepared by complex coacervation. Micrographs obtained by optical microscopy at 60X. Reproduced with permission from Reference [162].

Chitosan films exhibit low swelling in water, but membranes with diverse hydrophilic aptitudes can be prepared through the formation of mixtures or semi-interpenetrated and interpenetrated networks of chitosan with highly hydrophilic polymers like poly(vinyl alcohol), poly(vinylpyrrolidone) and gelatin [164]. PEC of chitosan with polyanions of natural origin like alginate, pectin or CMC or with synthetic ones, like poly(acrylic acid), have been investigated as matrices for controlled-release systems [165].

A pH-sensitive drug delivery system based on a glutaraldehyde crosslinked chitosan/gelatin hybrid network has been described. This gel swells at low pH and de-swells at high pH, thus exhibiting pH-dependent release of drugs [166].

Chitosan/polyethylene glycol/alginate miscrospheres have been proposed as good candidates for delivery of LMW heparin with antithrombotic properties [167]. Chitosan/CMC microcapsules of different compositions were prepared and tested as a protective matrix against the acid pH of the stomach for the oral administration of proteins and drugs [168]. Chitosan/xanthan microspheres are pH sensitive and biodegradable, and bestow a protective effect upon the drug in gastric and intestinal environment. They have also been proposed as a potential drug delivery system in the gastrointestinal tract [169].

Chitosan nanoparticles have been used for the nasal dosage of drugs and vaccines, since it has been demonstrated that they enhance the penetration of macromolecules through the nasal barrier [170].

Transdermal-drug delivery (TDD) devices using chitosan have been prepared for the permeation-controlled delivery of propranolol hydrochloride [171]. These chitosan-based TDD systems were composed of chitosan membranes with various crosslink densities as drug release controlling tools and chitosan gel as the drug reservoir. Drug release is highly affected by the crosslinking density of chitosan.

Chitosan has been considered a good candidate for non-viral gene delivery systems, since cationically charged chitosan can be complexed with negatively charged plasmid DNA. Self-aggregates of hydrophobically modified chitosan by deoxycholic acid (mean diameter of *ca.* 160 nm) can form charge complexes when mixed with plasmid DNA. These self-aggregate/DNA complexes are considered useful for the transfer of genes into mammalian cells *in vitro* and serve as a good delivery system [172].

25.7 APPLICATIONS IN AGRICULTURE

The utilization of chitin and chitosan in agriculture has followed four main directions: (a) protection of plants against plagues and diseases during pre-harvest and post-harvest, (b) promotion of antagonist microorganism action and biological control, (c) support of beneficial plant–microorganism symbiotic relationships and (d) plant growth regulation and development.

Chitin and its derivatives have been widely used for inducing defensive mechanisms in plants. The well-documented elicitor effect of chitin on plants confers them protection against many vegetable diseases [173]. Chitin and chitosan have fungicidal activity against many phytopathogenic fungi. The antiviral and antibacterial activity of chitosan and its derivatives have also been recognized [174].

These polysaccharides have been employed with success for controlling the parasitic nematodes in soils. The addition of chitin to the soil increases the population of chitinolytic microorganisms that destroy the eggs and cuticles of young nematodes which have chitin in their composition [175]. The chitinase and chitosanase activity in seeds protected by films of chitin and its derivatives has also been reported [176]. The antimicrobial properties of chitosan and its excellent film forming aptitude have been exploited in the post-harvest preservation of fruits and vegetables. Covering fruits and vegetables with a chitosan film imparts them antimicrobial protection and increased shelf life [177].

Chitin and chitosan in soil enhance plant–microorganism symbiotic interactions to the benefit of plants, as in the case of micorrizas. They also enhance the action of plague-controlling biological organisms such as *Tricoderma sp., Bacilus sp.* [178] and are good candidates for the encapsulation of biocides. Hence their efficiency in the control against pathogenic microorganisms and plagues is increased.

Chitosan and its derivatives induce favourable changes in the metabolism of plants and fruits. This results in an increased germination and higher crop yields [176, 179].

25.8 APLICATIONS IN THE FOOD INDUSTRY

Even though the use of chitosan as a food additive has not yet been approved by the FDA in the USA [180], nor by the Codex Committee on Food Additives in Europe, food is one of the industrial sectors in which chitosan has

drawn considerable attention in recent years [181]. The main applications of chitosan in food are summarized below with reference to selected examples.

25.8.1 Flocculation

At present, the use of chitosan in processed food is almost entirely limited to the treatment of liquid wastewaters [182]. Indeed, chitosan is an effective flocculant due to its capacity to associate with proteins, polysaccharides, lipids and pigments. The polycationic character of chitosan confers it the capacity to associate electrostatically with negatively charged molecules, such as proteins, phospholipids, carboxylic acids, etc. Therefore, chitosan has long been known to be a highly effective agent for the recovery of proteins [183] and phospholipids [184] from the whey produced in the manufacture of cheese. A preferential affinity for β-lactoglobulin and bovine serum albumin whey proteins was found using particles consisting of a chitosan–alginate PEC [185]. Other studies have demonstrated that chitosan can be used successfully to remove up to 99 per cent of emulsified lipids from wastewater produced in large amounts by palm oil-processing mills [186]. Some important factors that affect the level of lipids removal are the pH and the surface area of the chitosan substrate.

Yet another important area of application is the clarification and deacidification of fruit juices, for example, pineapple [187] and apple [188], as well as the adsorption of polyphenols (catechins, flavans, proanthocyanidins, cinnamic acids, etc.) in white wine, responsible for browning and maderization [189].

25.8.2 Antimicrobial activity

The well-established antimicrobial properties of chitin and chitosan against a wide spectrum of bacteria, fungi and viruses can lead to a potentially large reduction in the amount of synthetic food preservatives currently used. Although the precise mechanisms of antimicrobial action of chitin and chitosan are yet to be elucidated [35], there is a consensus that the interaction of chitosan with the negatively charged cell membranes, leading to the leakage of proteinaceous and other intracellular constituents, is determinant. As for the role of chitosan M_w, the experimental evidence is consistent with the fact that a low M_w enhances the antibacterial activity [190]. The most probable means of application are either through packaging wraps or directly as edible coatings for the preservation of fruits, vegetables and meat and fish products [191–196].

25.8.3 Other functional properties

The role of chitosan as a stabilizer of w/o/w is another of its potential usages as an industrial food additive [197]; this property has been attributed to a presumed amphiphilic character of chitosan and/or to the fact that it can increase the viscosity of the continuous aqueous phase. Additionally, the effectiveness of chitosan treatment on oxidative stability showed that the addition of chitosan at 1 per cent produced a decrease of 70 per cent in the 2-thiobarbituric acid (TBA) contents of meat after 3 days of storage at 4°C [198].

Another potential use of chitosan is for the improvement of the functional properties of fibril proteins of meat and fish products during frozen storage. Thus, the cryoprotective effect of chitosan isolated from various sources (DA in the range 0.14–0.17) has been evaluated, and a reduction in protein denaturation during storage at −20°C was demonstrated in mixtures of lizardfish (*Saurida wanieso*) myofibrillar protein containing 5 per cent chitosan [199].

25.9 APPLICATIONS IN COSMETICS

Because of its cationic character, chitosan interacts with negatively charged biological surfaces, such as skin and hair [200]. Other relevant characteristics of chitosan for cosmetic applications are its high molecular weight, water retention and film formation capacity as well as its heavy metal ion complexing ability.

High molecular weight chitosan decreases the loss of trans-epidermic water, increasing the humidity of the skin which preserves its softness and flexibility. Chitosan is an excellent ingredient for allergic skins and formulations with high molecular weight samples were found to reduce skin irritation [200]. Therefore it is very convenient to use chitosan in formulations containing alcohol, such as aftershave lotions and deodorants, again in order to reduce skin

irritation. Additionally, chitosan also inhibits inflammatory processes and promotes the regeneration of damaged tissues [201]. Sun-protecting emulsions that incorporate chitosan have a positive effect on water resistance, conferring an increased protection to the skin [202]. High molecular weight chitosan is a valuable component in deodorants because it absorbs humidity thus reducing transpiration. Besides, its antibacterial properties protect the skin [203].

When added to hair-care cosmetic preparations, chitosan interacts with keratin forming a uniform and elastic film which is more stable to high humidity than those usually formed with synthetic polymers. Moreover, it reduces the electrostatic charges, so that the hair does not stand on end, preserving the hair do. The hair treated with chitosan formulations has less tendency to adhere and is more easily brushed than with traditional fixers [204].

The bactericidal properties of chitosan prevent bad breath. Low molecular weight chitosan has also been shown to inhibit the oral adsorption of streptococci and have been proposed as a potential anticavity agent [205]. This makes chitosan a good additive in oral hygienic products such as tooth pastes, oral rinsing solutions and chewing gums.

25.10 OTHER APPLICATIONS

In addition to the already mentioned role of chitosan and some of its derivatives in the treatment of waste waters in the food industry, these substrates have also been applied for the treatment of residual waters from the mining and chemical industries, usually contaminated with heavy metals like mercury, cadmium, lead and copper. These processes can be very efficient, even if the metal content in the effluents is as low as 10–50 ppm [88].

Chitosan and chitin derivatives have been shown to increase the breaking strength and folding endurance of paper, without affecting its brightness. The water resistance of paper, as well as its sorption capacity for dyes, is also improved together with its electrical resistance [206].

Chitosan membranes have been successfully used in pervaporation processs, *e.g.* chitosan/poly(vinyl alcohol) (75:25 wt. per cent) blends showed excellent performance with good mechanical strength during the pervaporation dehydration of isopropanol [207].

Chitin and chitosan have been extensively employed for the immobilization of enzymes and cells applied in food technology and in biosensors [208]. Enzymes immobilized in these polymers possess an increased stability and are more temperature resistant. The immobilized enzyme can usually be recovered after reaction and reused. Immobilization is often achieved using glutaraldehyde as the crosslinking agent, although the enzymes have also been encapsulated by various techniques [209].

Other applications of chitin and chitosan include the spinning of fibres, preferably with the latter because of its higher solubility [223]; as additives in inks and dyes for the improvement of their rheological properties and colourfastness [224]; and in the coating of fabrics to control their shrinkage and enhance their dyefastness [223].

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