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Hemicelluloses: Major Sources, Properties and Applications

Iuliana Spiridon and Valentin I. Popa

ABSTRACT

This chapter deals with the major representatives of hemicelluloses, their properties and application. Hemicelluloses are plant cell wall polysaccharides that are not soluble in water, but they can be separated by aqueous alkali, or hydrolyzed by diluted acids. The main hemicelluloses include the following polysaccharides: xylan, glucuronoxylan, arabinoxylan, mannan, glucomannan and galactoglucomannan, and their representatives are different as a function of the plant species. The general structure of hemicelluloses is based on a sugar backbone substituted with side chains. The nature of the monosaccharide, as well as the linkages between structural units, determines some properties, such as solubility and the three-dimensional conformation of hemicelluloses. At present, there is an increasing interest to develop new applications of hemicelluloses as raw materials for chemical industry and also in the fields of food and pharmaceutical industries.

Keywords

Hemicelluloses, Polysaccharides, Xylan, Glucuronoxylan, Arabinoxylan, Mannan, Glucomannan and galactoglucomannan, Polymer, Biosynthesis, Biodegradation, Properties, Applications

13.1 STRUCTURE, SOURCES AND PROPERTIES

Hemicelluloses are the second most abundant polysaccharides in nature after cellulose. They occur in close association with cellulose and lignin and contribute to the rigidity of plant cell walls in lignified tissues. Hemicelluloses constitute about 20–30 per cent of the total mass of annual and perennial plants and have a heterogeneous composition of various sugar units, depending on the type of plant and extraction process, being classified as xylans (β-1,4-linked D-xylose units), mannans (β-1,4-linked D-mannose units), arabinans (α-1,5-linked L-arabinose units) and galactans (β-1,3-linked D-galactose units).

In Table 13.1 gives the main hemicelluloses of hardwood and softwood.

Xylan [1] is one of the major constituents (25–35 per cent) of lignocellulosic materials. Its structure has a linear backbone consisting of β-1,4-linked D-xylopyranose residues. These may be substituted with branches containing acetyl, arabinosyl and glucuronosyl residues, depending on the botanic source and method of extraction.

Xylans are the main hemicelluloses in hardwood and they also predominate in annual plants and cereals making up to 30 per cent of the cell wall material. Hardwood xylan (*O*-acetyl-4 methyl-glucuronoxylan) is substituted at irregular intervals (Fig. 13.1) with 4-*O*-methyl-α-D-glucuronic acid groups joined to xylose by α-1,2-glycosidic linkages. On an average, every tenth xylose unit has an uronic acid group attached at C2 or C3 of the xylopyranose.

Barley arabinoxylans are composed of D-xylopyranosyl (Xyl) units linked by β-(1,4) bonds with single L-arabinofuranosyl residues connected to the backbone of one or both of the α-(1,2) or α-(1,3) bonds [2, 3] Another unique

Table 13.1

The main hemicelluloses in wood

	Hardwood	Softwood
Methylglucuronoxylan	80–90	5–15
Arabinomethylglucuronoxylan	0.1–1	15–30
Glucomannan	1–5	1–5
Galactoglucomannan	0.1–1	60–70
Arabinogalactan	0.1–1	1–15
Other galactans	0.1–1	0.1–1
Pectins	1–5	1–5

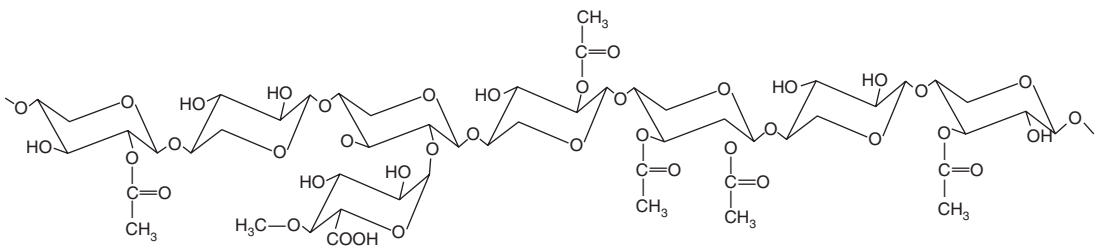

Figure 13.1 Structure of *O*-acetyl-(4-OMe-glucurono)xylan from hardwood.

feature of barley, wheat, and rye arabinoxylan, is the presence of ferulic acid that is covalently linked to the arabinose residue *via* an ester bond [4].

The physicochemical characteristics and functional properties of carbohydrate macromolecules depend on their molecular mass, degree of polymerization, branching and macroscopic structure.

Arabinoxylan or β-(1,4)-xylan polymers in an unsubstituted form tend to aggregate into insoluble complexes that are stabilized by intermolecular hydrogen bonds [5]. The polymer forms a three-fold, left-handed helix, which in the solid state appears as an extended twisted ribbon [6]. Unsubstituted polymer regions exhibit a tendency for interchain aggregation through H-bonding, which is the most likely reason why these polymers are less soluble in water. The solubility of arabinoxylan macromolecules is improved by the presence of arabinose substituents that prevent intermolecular aggregation of unsubstituted xylose residues [7]. The removal of the arabinofuranosyl residues is induced by the action of α-L-arabinofuranoside arabinofuranohydrolases (EC 3.2.1.55), enzymes which have broad specificities for the various (1 → 2)-, (1 → 3)- and (1 → 5)-α-L-arabinofuranosyl linkages of arabinan and arabinoxylans [8].

The molecular structure of sycamore xyloglucan was characterized by methylation analysis of the oligosaccharides obtained by endoglucanase treatment of the polymer. It was found that polymer structures were based on a repeating heptasaccharide unit, which consists of four residues of beta-1–4-linked glucose and three residues of terminal xylose, a single xylose residue being glycosidically linked to carbon 6 of three of the glucosyl residues [9].

Appreciable amounts of xylan, the main component of plant hemicelluloses, are currently not exploited to their full extent. Xylan and xylooligosaccharides can be converted to xylose by chemical or enzymatic hydrolysis. Subsequently, xylose can be converted biologically to single cell proteins and to a whole range of fuels and chemicals, and can also be used as a carbon source for the production of glucose isomerase. Thus, the most promising approach of economically feasible processes of xylan bioconversion is the use of microorganisms able to transform xylan directly to single cell proteins, fuels and chemicals. Microorganisms such as *Fusarium oxysporum, Aureobasidium pullulans* and *Candida shehatae* are able to transform xylose to ethanol. For the direct xylan bioconversion only anaerobic thermophilic bacteria and a few representative fungi and yeasts combine the two metabolic pathways necessary for direct xylan bioconversion [10, 11].

In the softwood, galactoglucomannan (Fig. 13.2) is the most abundant hemicellulose. Its backbone is constituted by β-1,4-linked D-glucopyranose D-mannopyranose units randomly distributed in the main chain. Partial substitutions are

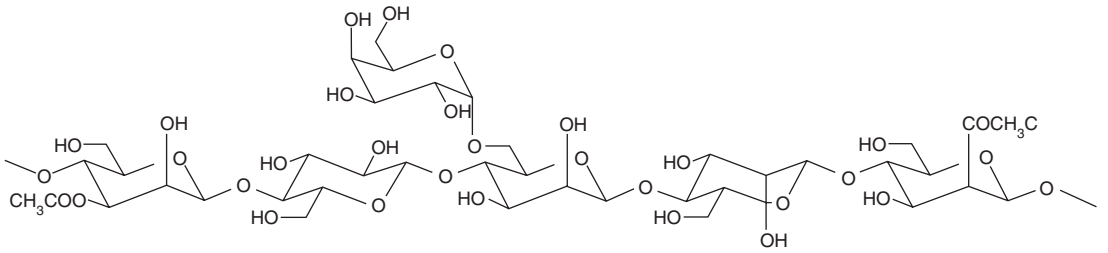

Figure 13.2 Structure of *O*-acetyl-galactoglucomannan.

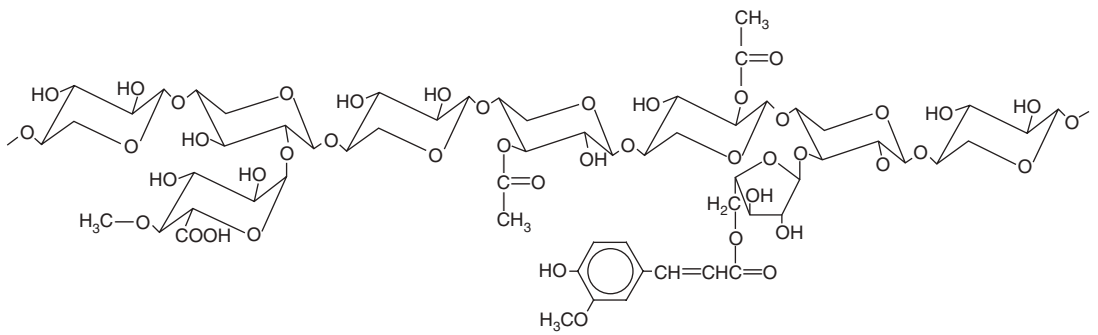

Figure 13.3 Structure of xylan from annual plants.

possible with α-D-galactose side groups, which can be attached to both mannose and glucose units by an α-1,6-linkage [12, 13]. Depending on the source of the polysaccharide, mannose/galactose ratio can vary from 1.0 to 5.3 [14, 15].

Larch arabinogalactan has a backbone of (1–3)-linked β-D-galactopyranosyl units each of which contains a side chain at position C-6. Most of these side chains are galactobiosyl units containing a (1–6)-β-D-linkage. Another side chain type that occurs is a single L-arabinofuranose unit or 3-O-(β-L-arabinopyranosyl)-α-L-arabinofuranosyl units. The preliminary X-ray fibre diffraction data [16] proved that western larch arabinogalactan can adopt a triple helix conformation.

In annual plants, the main hemicelluloses are represented by xylans (Fig. 13.3), which are more heterogeneous than the xylans from wood tissues [17]. They contain both glucuronic acid and/or its 4-*O*-methyl-ether and arabinose attached to C2 or C3 of the xylose units. Both xylan and glucomannans can be partly acetylated [18].

Arabinoxylans consist of α-L-arabinofuranose residues attached as branch-points to β-(1 → 4)-linked D-xylopyranose polymeric backbone chains. These may be 2- or 3-substituted or 2- and 3-disubstituted. In wheat flour, the distribution of the type of substitution is not random, but that of the substituted residues along the chain occurs randomly [19]. The arabinose residues may also be linked to other groups, such as glucuronic acid residues and ferulic acid crosslinks [20]. It was found that the molecules take up a twisted ribbon conformation with a three-fold symmetry. The free molecules in solution may, however, take up a wide variety of conformations, with only moderately extended structures. Although the backbone xylan structure is similar to that occurring in cellulose, there is little driving force to produce crystalline type structures as the intra- and inter-molecular hydrogen bonds associated with the 6-hydroxyl groups are necessarily absent. The side chains of arabinose reduce the interactions between chains, because of their inherently more flexible water-hungry furanose conformations. Arabinose and xylose can usually be removed by enzymatic hydrolysis with cellulases and hemicellulases [21].

The addition of the β-xylosidase and α-L-arabinofuranosidase enzymes to purified xylanases, more than doubled the degradation of xylan from 28 to 58 per cent of the total substrate, with xylose and arabinose being the major sugars produced [22]. The presence of all three purified enzymes resulted in the production of xylose, arabinose and substituted xylotetrose. β-xylosidase and α-L-arabinofuranosidase display a synergysm with endoxylanase with respect to xylan degradation. Endoxylanases generate free chain ends upon which the β-xylosidase can act, while the debranching activity of α-L-arabinofuranosidase removes the substitute arabinose that might otherwise block the enzyme's progress [22].

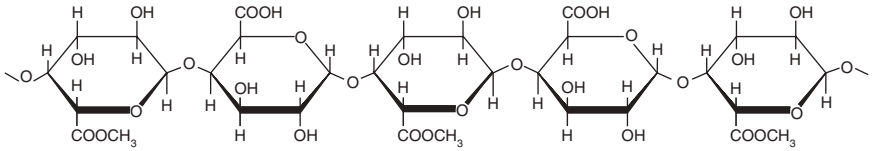

Figure 13.4 The main component of pectin.

The arabinoxylan from *Plantago ovata* is resistant against enzymatic attack. The unusual physicochemical and physiological properties of arabinoxylan may be explained directly from the polysaccharide structure which involves a β-(1 → 3)-linked xylose disaccharide attached at the 2-position to the xylan backbone and has been found to interact strongly with neighbouring arabinose residues and to introduce a conformational lock (or 'kink') into the structure, which may be released with a rise temperature [23].

Other hemicelluloses such as arabinans, galactans, arabinogalactans, rhamnogalactans, etc are regarded as pectic substances of plant cell walls. They are a complex mixture of colloidal polysaccharides, more abundant in the soft tissues of some fruit, as well as in sugar beet pulp, but less frequent in wood tissues.

Pectins (Fig. 13.4) form a hydrated crosslinked three-dimensional network in the matrix of the primary cell walls and play diverse functions in the cell physiology, growth, adhesion and separation [24]. The gel-like property of the cell wall is derived in part from pectins.

The biogenesis of pectins proceeds during deposition on the P-wall. At the same time, labelled representatives of this series, namely, glucose, glucuronic acid and xylose, appear indicating that the epimerase in the Golgi vesicles no longer quantitatively transforms the resorbed glucose into galactose. Later on, the deposition of the S-wall takes place and the activity of the epimerase is stopped, so that the pectic substances, indispensable for the extension growth of the P-wall, are replaced by hemicellulosic substances such as polyglucuronic acids and xylans.

The conversion of hexose into uronic acid and pentose units of cell wall polysaccharides in higher plants may occur, not only by the sugar nucleotide oxidation pathway, which leads to UDP-uronate, but also by the myoinositol oxidation pathway. In this case, the homocyclic inositol ring is split, oxidized at C(6) and concurrently replaced by a heterocycle with an —O—bridge between C(1) and C(5), as shown by label experiments. Glucuronic acid can be generated in this way.

Pectic substances are complex colloidal acid polysaccharides with a backbone of galacturonic acid residues linked by α-1,4-glycosidic linkages. The side chains of the pectin molecule consist of L-rhamnose, arabinose, galactose and xylose [25]. D-Galacturonic acid is the principal monosaccharide unit of pectin. The D-galacturonic acid residues are linked together by α-1,4-glycosidic linkages. The molecular mass of pectin ranges from 50 000 to 150 000. The carboxyl groups of galacturonic acid are partially esterified by methyl groups and are partially or completely neutralized by sodium, potassium or ammonium ions. Based on the type of modification, different pectic substances exist in nature. Thus, a pectin in which more than 50 per cent of the galacturonic acid residues are esterified is called high methoxyl or HM pectin, whereas a pectin in which this extent of esterification is less than 50 per cent, is called low methoxyl or LM pectin. Homogalacturonans with a linear structure make up the 'smooth region' of pectin, while the branched polysaccharides – rhamnogalacturonans and other pectin structures such as xylogalacturonan [26] and α-(1,4)-linked D-galacturonic acid substituted with β-D-xylose at the C3 position [27] – make up its 'hairy region'.

Because the predominant cation in the cell wall is the calcium ion, its middle lamella consists largely of calcium pectate. Calcium ions are involved in the stabilization of the cell wall structure by crosslinking pectin chains, the ion exchange processes and the control of the activities of the wall enzymes. An 'egg-box' model was proposed for the calcium coordination in the middle lamella, in which two helical 'polygala' fragments are mutually attracted via ionic interactions and coordination of calcium ions between chains. In these dimers, the calcium ions are sandwiched between the inner faces of both monomeric components on the specific sites [28]. Calcium egg boxes, crosslinking smooth regions in pectin chains, probably play an important structural role in keeping middle lamellas together in many non-lignified tissues.

Agar is obtained from the family of red seaweeds (*Rhodophycae*) as the carrageenans. Carrageenan compounds differ from agar in that they have sulphate groups (—OSO_3^-) in the place of some hydroxyl groups. Commercially, agar is produced from species of *Gelidium* and *Gracilariae*[29, 30]. Agar (Fig. 13.5) is insoluble in cold water but dissolves in boiling water to give random coils. Gelation is reported to follow a phase separation process including association on cooling (about 35°C), forming gels with up to 99.5 per cent water and remaining

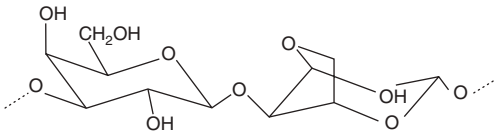

Figure 13.5 Structure of agar repeat unit.

solid up to about 85°C, although these findings have been disputed. Agar consists of a mixture of agarose and agaropectin [31, 32].

Agarose is a linear polymer with a molecular mass of about 120 000. The agarose gel contains double helices, stabilized by the presence of water molecules bound inside the double-helical cavity. It seems that this network model is excessive and that only a small proportion of double-helical junction zones would be sufficient to realize the gelation process. This is why some authors consider that network structure of agarose gel consists of single chain [33, 34].

Agaropectin is a heterogeneous mixture of smaller molecules that occur in lesser amounts. Their structures are similar, but slightly branched and sulphated, and may have methyl and pyruvic acid ketal substituents. They gel poorly and may be easily removed from the agarose molecules using their charge. The quality of agar is improved by an alkaline treatment that converts L-galactose-6-sulphate into 3,6-anhydro-L-galactose.

Another polysaccharide extracted from seaweeds is carrageenan. Carrageenans are linear, water-soluble polymers that form solutions, whose viscosity depends mainly on the concentration of the polymer, the temperature of the solution and the type of carrageenan. Chemically, they are highly sulphated galactans and, because of their half-ester sulphate moieties they are strongly anionic polyelectrolytes. In this respect, they differ from agars and alginates, the other two classes of commercially exploited seaweed hydrocolloids. Carrageenans are susceptible to depolymerization through acid-catalyzed hydrolysis. High temperatures and low pHs lead to a complete loss of functionality. Their rate of hydrolysis at a given pH and temperature is markedly lower if they are in the gel, rather than the sol state. This can be achieved by ensuring that gel-promoting cations are present in a sufficient concentration to raise the gel melting temperature above the temperature at which they will be treated. Carrageenans can be used in acidic media, provided, however, that they are not subjected to prolonged heating.

Carrageenan is frequently preferred over other thickening, suspending and binding ingredients, because it is natural, economical, readily available and functional in an extremely broad application base. Carrageenans can be produced *via* a variety of process techniques such as alcohol extraction, potassium chloride gel press or extraction with various alkalis. The process technique is important, because it influences the gel characteristics. Likewise, different seaweeds also influence the gel characteristics.

The three preferred carrageenan types are *kappa*, *iota* and *lambda*. The *kappa* and *iota* types only dissolve in a heated water medium, whereas the *lambda* type is soluble in cold water. Gels created from different carrageenan types may be fluid, elastic or rigid and are heat-reversible. Gelling temperatures and gel strength are also influenced by added ingredients such as salts and proteins.

Alginate is a gelling polysaccharide extracted from seaweeds. Most of the large brown seaweeds are potential sources of alginate, their properties being different from one species to another. The main commercial sources are species of *Ascophyllum, Durvillaea, Ecklonia, Laminaria, Lessonia, Macrocystis, Sargassum* and *Turbinaria*. The monomeric units of alginates, α L-guluronic acid residues (Fig. 13.6) and β D-mannuronic acid (Fig. 13.7) are C5 epimers of each other.

Alginates are not random copolymers, consisting instead of blocks of similar and strictly alternating residues, each of which has different conformational preferences and behaviour. Thus, poly β-(1 → 4)-linked D-mannuronate prefers forming a three-fold left-handed helix with (weak) intramolecular hydrogen bonding between the hydroxyl group in the 3-position and the subsequent ring oxygen, while poly α-(1 → 4)-linked L-guluronate forms stiffer (and more acid-stable)

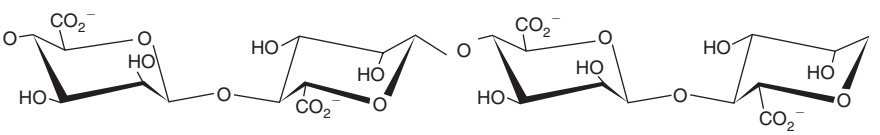

Figure 13.6 Structure of β-D-mannuronic acid.

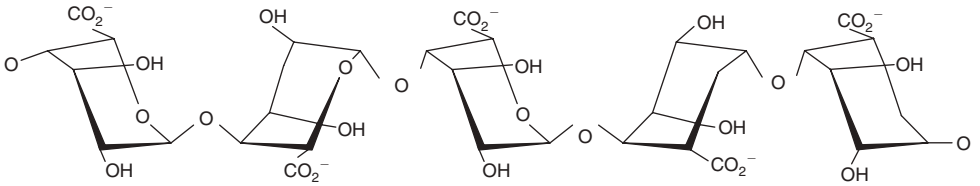

Figure 13.7 Structure of α-L-guluronic acid residues.

two-fold screw helical chains, preferring intramolecular hydrogen bonding between the carboxyl group and the 2-OH group of the prior residues and the 3-OH group of the subsequent residues, the latter interaction being weaker.

It has been shown that the polymer chain is made up of three kinds of regions or blocks. The G blocks contain only units derived from L-guluronic acid, the M blocks are based entirely on D-mannuronic acid, while the MG blocks consist of alternating units of the two acids. In practice, an alginate molecule can be regarded as a block copolymer containing M, G and MG blocks, whose proportion varies as a function of the seaweed source and influences the physical properties of the corresponding alginates [35, 36]. Thus, the gel formation by addition of calcium ions, involves the G blocks so that the higher their proportion, the greater the gel strength, whereas the solubility of alginates in an acid medium, depends on the proportion of MG blocks. The monovalent cation salts [Na^+, K^+, NH_4^+, $(CH_2OH)_3NH^+$] of alginic acid, and its propylene glycol ester, dissolve in water, but alginic acid and its calcium salt do not. Neutral alginate solutions of low-to-medium viscosity can be kept at 25°C for several years, without any appreciable viscosity loss, as long as a suitable microbial preservative is added. Solutions of highly polymerized alginates will lose viscosity at room temperature within a year and in order to achieve high, stable viscosities, it is better to add calcium ions to a solution of an alginate with a moderate molecular weight. All alginate solutions depolymerize more rapidly as the temperature is raised. Alginates are most stable in the PH range of 5–9.

β-glucans were identified as major component in the bran of *Gramineae* (such as barley, oats, rye and wheat, consisting of linear unbranched polysaccharides of linked β-(1 → 3)- and β-(1 → 4)-D-glucopyranose units [37]. β-Glucans form 'worm'-like cylindrical molecules containing up to about 250 000 glucose residues that may produce crosslinks between regular areas containing consecutive cellotriose units. They form thermoreversible infinite network gels. Ninety per cent of the β-(1 → 4)-links are in cellotriosyl and cellotetraosyl units joined by single β-(1 → 3)-links, with no single β-(1 → 4)- or double β-(1 → 3)-links. The ratio of cellotriosyl/cellotetraosyl is between 2.0 and 2.4 in oats, about 3.0 in barley and about 3.5 in wheat. The high molecular mass β-glucans from barley and oats are viscous due to labile cooperative associations, whereas lower molecular mass β-glucans can form soft gels as the chains are easier to rearrange to maximize linkages. Barley β-glucan is highly viscous and pseudoplastic, both properties decreasing with increasing temperature.

The related hydrocolloid curdlan has only β-(1 → 3)-D-glucopyranose units which form thermoreversible triple-helical structures on heating and become irreversibly linked as the concentration or temperature is increased. Curdlan is a microbial fermentation extracellular polymer produced by a mutant strain of *Alcaligenes faecalis* var. *myxogenes* [38]. Curdlan was also produced by pure culture fermentation using *Agrobacterium radiobacter* NCIM 2443 using glucose, sucrose, maltose as carbon sources, sucrose being the most efficient [39, 40]. It is insoluble in water, which limits its biological applications. Water insolubility is generally attributed to the existence of extensive intra/intermolecular hydrogen bonds.

Curdlan has a moderate molecular mass (DP ∼ 450) being constituted from unbranched linear 1 → 3 β-D-glucan with no side chains (Fig. 13.8). It has junction zones consisting of parallel in-phase triple right-handed six-fold helices (fibre repeat 18.78 Å) forming an uncharged rigid rod-like conformation [41]. The chains are held by intrahelix hydrogen bondings between the 2-OH groups, each formed by donation to one chain and acceptance from the other on the inside of the helix axis. As single stranded curdlan forms a six-fold helix stabilized by a chain of intramolecular hydrogen bonds between neighbouring 2-OH groups, the change from single to triple helices involves these 2-OH groups changing their hydrogen bonding allegiance from intramolecular to intermolecular.

Okuyama *et al.* [42] proposed the single right-handled six-fold helical conformation in the highly hydrated crystal, in which the water content was more than 70 wt per cent in the unit cell. This single helix is stabilized by weak intramolecular hydrogen bonds between O(5) and O(4) of the next glucose residue (distance 0.314 nm).

Curdlan gum is tasteless and produces retortable freezable food elastic gels. It is insoluble in cold water, but aqueous suspensions plasticize and briefly dissolve before producing irreversible gels (*i.e.* curdling, hence its

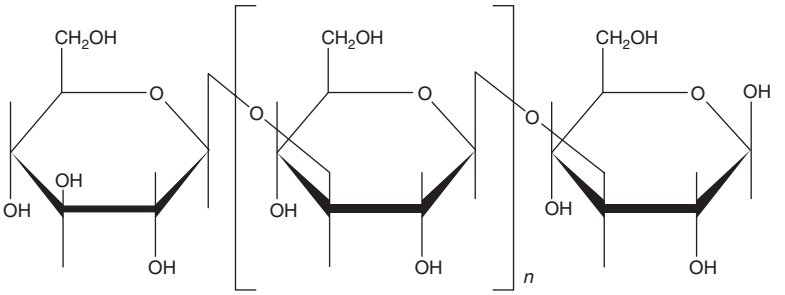

Figure 13.8 Structure of curdlan.

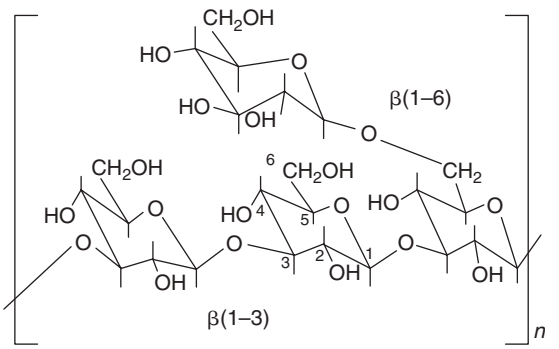

Figure 13.9 Structure of scleroglucan.

name) on heating to around 55°C, which then remain on cooling. Heating at higher temperatures produces more resilient gels by the aggregation of the triple-helical structures and synaeresis. The 'curds' consist of mixtures of single and triple helices [43–45].

Scleroglucan (from *Sclerotinia sclerotiorum*) is a 1 → 3 β-D-glucan with additional 1 → 6 β-links (Fig. 13.9) that confer water solubility to it under ambient conditions, but do not significantly interfere with a triple helix gelling process, similar to that of curdlan [46]. Similar polysaccharides can also be extracted from other sources such as waste yeast.

Scleroglucan presents a triple-helical backbone conformation [47, 48] and in solution the chains assume a rod-like triple-helical structure [49] in which the D-glucosidic side groups are on the outside and prevent the helices from coming close to each other and aggregating.

Pullulan (Fig. 13.10) is one of the few neutral water-soluble microbial polysaccharides that can be obtained in large quantities by fermentation [50, 51]. Pullulan biosynthesis is accomplished through mediation of sugar nucleotide–lipid carrier intermediates associated with the cell membrane fraction [52]. It is an extracellular, unbranched homopolysaccharide consisting of maltotriose and maltotetraose units with both α-(1 → 6) and α-(1 → 4) linkages,

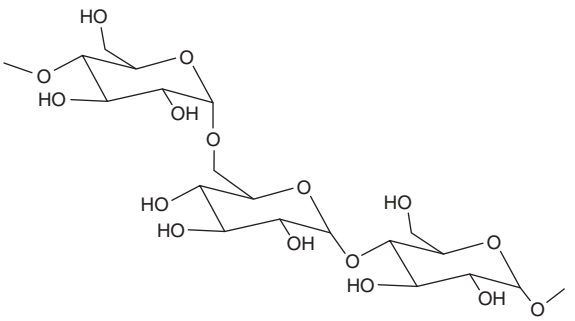

Figure 13.10 Structure of pullulan.

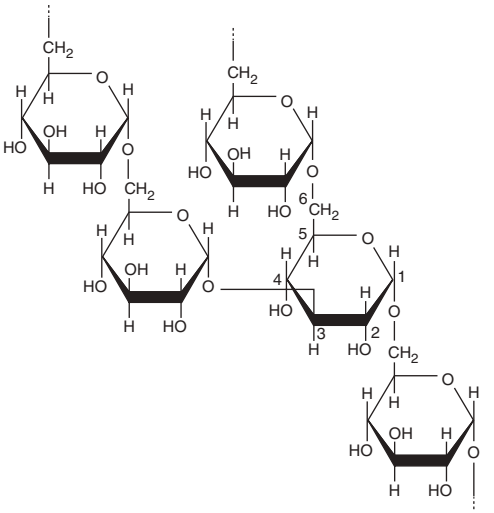

Figure 13.11 Structure of dextran.

whose molecular mass depends on the culture conditions and strain [53–55]. It adopts a random coil-type structure in aqueous solution, generates a Raman optical spectrum that closely resembles that of D-maltotriose [56]. The alternation of α-1 → 4 and α 1 → 4 is regular, resulting in structural flexibility and enhanced solubility [57].

Pullulan produces high-viscosity solutions at relatively low concentration and can be utilized to form oxygen-impermeable films, thickening or extending agents and adhesives. Pullulan has many uses as an industrial plastic. It can be processed into compression mouldings that resemble polystyrene or polyvinyl chloride in transparency, gloss, hardness, strength and toughness, but is far more elastic. It decomposes above 200°C without the formation of toxic gases.

Dextran is a microbial biopolymer [58] whose molecular structure is composed exclusively of monomeric 2-D-glucopyranosil units, linked mainly by (β-1,6) glucosidic bonds (Fig. 13.11). Its applications depend on its molecular mass [59]. Two dextran products are available in most countries for clinical purposes: dextran 70, with a molecular mass of about 70 000 and dextran 40 with a molecular mass 40 000. Dextran fractions are readily soluble in water and electrolyte media to form clear, stable solutions significantly insensitive to pH. They are also soluble in some solvents such as methyl sulphide, formamide and ethylene glycol [60].

Xanthan, a microbial polysaccharide produced by the *Xanthomonas* bacterium, represents a great scientific and industrial interest due to its properties. It is a heteropolysaccharide with a high molecular mass, between 0.9 and 1.6×10^6, as a function of the microbial source and fermentation conditions [61–63]. The pentasaccharide repeating unit (Fig. 13.12) is assembled on an isoprenoid lipid carrier by sequential addition of individual sugar residues that are donated by sugar nucleotide diphosphate precursors. Each sugar addition is catalyzed by a specific glycosyltransferase enzyme. The mannose residues of the repeating units are specifically acetylated and pyruvylated. The repeating unit is polymerized, and the polymer is subsequently secreted [64, 65].

In its native state, xanthan is a right extended chain possessing a helical conformation [66], in the solid state this polysaccharide has a S1 helix conformation [67], while the xanthan crystal chain takes an antiparallel right-handed five-fold (5/1) double helix that is stabilized by four intramolecular and one intermolecular hydrogen bonds.

In solutions of low ionic strength or at high temperature, the xanthan gum chains adopt a random coil configuration, since the anionic side chains repel each other. The addition of electrolytes reduces the electrostatic repulsion among the side chains, allowing them to wrap around and hydrogen bond to the backbone. The polymer chain straightens into a relatively rigid helical rod. This shape tends to revert to the random coil if the gum solution is highly diluted or heated. With increasing electrolyte concentration, however, the rod shape is maintained at higher temperatures and greater dilutions [68].

Gellan is an extracellular heteropolysaccharide produced by *Sphingomonas paucimobilis*, previously known as *Pseudomonas elodea.* It is composed of linear tetrasaccharide repeating units of β-D-glucose, β-D-glucuronic acid, β,-D-glucose and α-L-rhamnose (Fig. 13.13). Occasionally, it carries acetyl and glyceryl groups on the glucose units [69, 70].

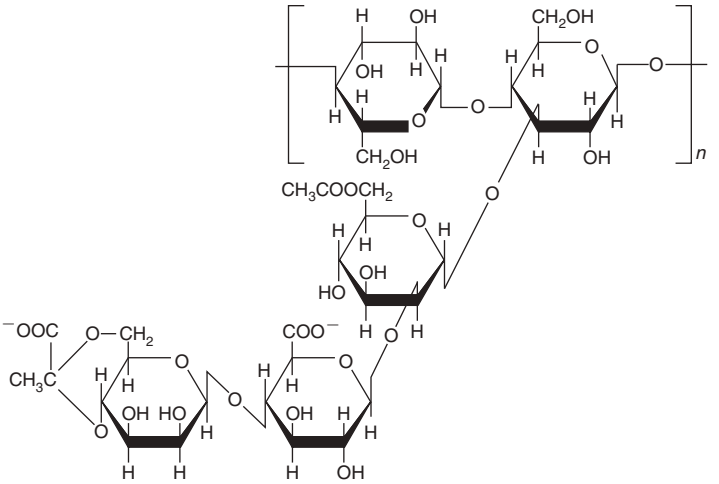

Figure 13.12 Structure of xanthan.

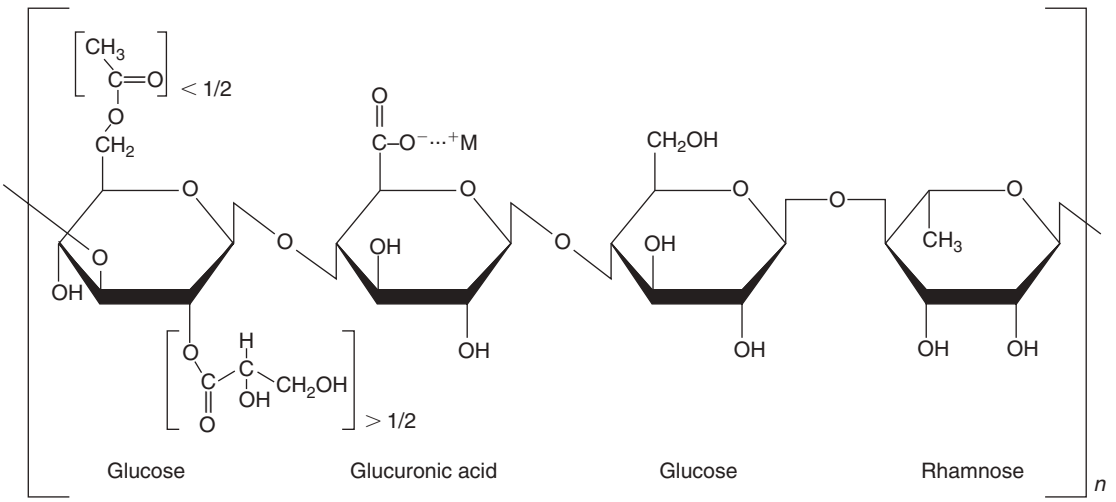

Figure 13.13 The repeat units of gellan.

The degree of ester substitution directly influences the gellan properties in solution and gel [71]. Aqueous solutions of gellan are highly viscous and show high thermal stability. The gellan gum exhibits good stability over a wide pH range of 3.5–8.0. Fibre diffraction analysis [72] showed that the two left-handed, three-fold helical chains are organized in parallel fashion in an intertwined double helix and that the duplex is stabilized by interchain hydrogen bonds at each carboxylate group.

The properties of gellan can be easily modified. A hot caustic treatment yields a polymer that has the desirable characteristic of low viscosity at high temperature. Cooling gellan in the presence of various cations (*e.g.* calcium) results in the formation of strong gels. In a heated gellan solution, the polymer chains exist as disordered coils. Cooling without the presence of metal cations leads to the formation of weak, thermoreversible interactions, such as hydrogen bonding and van der Waals attractions between adjacent polymer chains. However, with the introduction of metal cations, the interactions formed with the carboxyl groups are much stronger and more stable. Monovalent cations form stiff gels, perhaps allowing for more hydrogen bonding to occur between the polymer helices, also leading to aggregation and gel formation. Divalent cations may bridge two adjoining carboxylic acid sites, thus causing local aggregation of the helices to make a more rigid gel.

13.2 APPLICATIONS

Arabinogalactans are used as emulsifiers, stabilizers and binders in the food, pharmaceutical and cosmetic industries. Arabinogalactan has properties that make it suitable as a carrier for delivering diagnostic or therapeutic agents to hepatocytes via the asialoglycoprotein receptor [73]. Experimental studies indicated that larch arabinogalactan can stimulate natural killer cell cytotoxicity, enhance other functional aspects of the immune system, and inhibit the metastasis of tumour cells to the liver. These immune-enhancing properties also suggest an array of clinical uses, both in preventive medicine, due to its ability to build a more responsive immune system, and in clinical medicine, as a therapeutic agent in conditions associated with a lowered immune function, decreased NK activity or chronic viral infection [74].

The xylans have numerous medical applications [75, 76]. The films based on glucuronoxylan (isolated from aspen wood) and xylitol or sorbitol show low oxygen permeability and thus have a potential application in food packaging [77].

Pectin is widely used in the food industry as a gelling agent to impart a gelled texture to foods, mainly fruit-based foods such as jams and jellies. Thus, LM pectins (<50 per cent esterified), form thermoreversible gels in the presence of calcium ions and at low pH (3–4.5) whereas HM pectins rapidly form thermally irreversible gels in the presence of sufficient (*e.g.* 65 per cent by mass) sugars, such as sucrose and at low pH (<3.5); the lower the methoxyl content, the slower the set. Pectin is also used for the stabilization of low-pH drinks, including fermented drinks and mixtures of fruit juice and milk. In the medical field, pectin is used in combination with the clay kaolin (hydrated aluminium silicate) against diarrhoea [78]. It is also used as a component in the adhesive part of ostomy rings. Pectin is moreover marketed as a nutritional supplement for the management of elevated cholesterol [79, 80]. Finally, pectin has been found to alter the characteristics of the fibrin-network architecture, suggesting that it may have some antithrombotic effects [81].

Agar is used in the food industry in icings, glazes, processed cheese, jelly sweets and marshmallows and sometimes as a substitute for gelatin. Another interesting application of agar comes from the fact that it constitutes a very good microbiological medium [82].

Carrageenans and semi-synthetic sulphated polysaccharides, like laminarin sulphate, were shown to be potent angiogenesis inhibitors [83].

Alginates generally show high water absorption and may be used as low viscosity emulsifiers and shear-thinning thickeners. They can be used to stabilize phase separation in low-fat fat-substitutes, for example as alginate/caseinate blends in starch three-phase systems. Alginate is used in a wide variety of foodstuff such as pet food chunks, onion rings, stuffed olives, low-fat spreads, sauces and pie fillings [84]. Propylene glycol alginates have widespread use as acid-stable stabilizers for, for example, preserving the head on beers [85, 86]. Alginates are suitable for various potential clinical applications, such as cell encapsulation, drug delivery and tissue engineering [87, 88]. Thus, numerous studies [89–91] analyzed the biocompatibility of alginates/sodium alginate and drugs, which are the most important factors affecting the drug release from matrix tablets, or their effects on cell proliferation, cell migration etc. [92]. Because the properties of alginate hydrogels are readily controlled with the chemical structure of the sugar residues and different crosslinked molecules, and these materials interact with cells [93], they are used to engineer bones [94], cartilage [95], muscles, blood vessels [96], liver and nerve tissues [97, 98]. Finally, alginate was used as a scaffold to transplant subcultured human dental pulp cells subcutaneously into the backs of nude mice, and it was proved that subcultured dental pulp cells actively differentiated into odontoblast-like cells and induced calcification in an alginate scaffold [99].

β-glucans have important health benefits, especially in coronary heart disease, cholesterol lowering and reducing the glycemic response. These positive effects are linked to their high viscosity, although it may be that some of them arise from appetite suppression. Almost all carboxymethyl glucans possess properties useful in cosmetic and dermatological applications [100]. β-glucans were identified in different species of mushrooms and when isolated from the mature fruiting bodies of *Agaricus* species they displayed their potential therapeutical benefits [101–103].

Another β-glucan from yeast (*Saccharomyces cereviseae*) is recognized for its ability to activate nonspecific immune response [104–106].

Curdlan, at concentration between 0.05 per cent and 3 per cent, improves the texture, palatability, stability, water binding and holding of food. It can be used in fat replacement because consumers want healthier products with fewer calories, but they are unwilling to give up the mouth feel and flavour characteristics of full-fat products. When hydrated and heated, curdlan's particles mimic the mouth feel of fat containing products. Curdlan also binds the additional water in the system, so that it becomes unavailable for ice crystallization and moisture migration upon storage, freeze-thawing, or deep-fat frying. In addition, aqueous suspensions of curdlan exhibit thixotropic

properties that are very important in mimicking the rheological behaviour of fats in processed liquid foods such as low-fat dressings, sauces and gravies [107, 108]. Curdlan of *Alcaligenes faecalis* was reported to display an anti-tumour activity [109, 110], while the sulphated curdlan derivative showed *in vitro* a beneficial effect for HIV-1 treatment [111, 112]. Curdlan is also effective in the prevention and treatment of ischaemic cerebrovascular disease [113]. It has been proved that laminarin from *Eisenia bicyclis* stimulates monocytes to inhibit the proliferation of U 937 cells [114, 115]. Its elicitors may be good candidates as fungicide disease control, because they are natural products and recyclable in the ecosystem [116, 117].

In the cosmetic industry, scleroglucan may be used in various skin care preparations, creams and protective lotions, while in pharmaceutical products, it may be used as a laxative, in tablet coatings and in general to stabilize suspensions [118]. Scleroglucan also proved to have an immunomodulating effect, with numerous medical applications [119–121].

Pullulan can be modified hydrophobically by the reaction with dodecanoic acid, by modulating the polymer-to-acid ratio. It was found that the addition of sodium dodecyl sulphate, alone or in mixtures with other surfactants, resulted in the formation of micelles. The long foam lifetime of these hydrophobically derivatized pullulans is suitable for applications in mineral flotations and in biomedicine (vesicle coating) [122]. Films formed from pullulan are suitable for coating foods and pharmaceuticals [123–125] and prevent oxidation, especially when the exclusion of oxygen is desirable. Pullulan also finds medical applications in vaccine production and as a plasma extender [126, 127]. Ester and ether derivatives of pullulan have adhesive qualities similar to those of gum arabic, their viscosity and adhesive properties being dependent on the degree of polymerization of the polysaccharide. Finally, modified pullulan can be used as a stationary paste that gelatinizes upon moistening.

Dextran–hemoglobin compounds may be used as blood substitutes that have oxygen delivery potential and can also function as plasma expanders. Dextran hydrogels [128] can be used to engineer tissues or to control drug delivery. Blends, based on dextran [129, 130] as well as dextran derivatives [131], have medical applications. Thus, diethylaminoethyldextran, a polycationic derivative of dextran, is recommended for numerous applications in molecular biology and the health-care sector. Applications of dextran derivatives in gene therapy have also been reported [132, 133]. Dextran sulphate is an inhibitor of the human immunodeficiency virus (HIV), binding to T-lymphocytes, but because of its low oral bioavailability, it has not been used therapeutically [134–136]. Other modified dextrans such as Sephadex@ are used extensively in the separation of biological compounds. Finally, iron dextran solutions are used for the treatment of human and veterinary anaemic iron deficiency.

Xanthan can form gels either by crosslinking with certain metal ions or by the interaction with other polysaccharides. These gels are used in the oil industry to modify the permeability profiles of heterogeneous oil reservoirs. The high solution viscosity, compatibility with salts and stability to shear and heat makes xanthan solutions well suited to meet the requirements of polymer flooding systems. In the food industry, xanthan is used in products such as beverages, desserts and dressings, but also as a gelling agent for cheese spreads, ice creams and puddings. Moreover, it can stabilize emulsified creams with pharmaceutical and cosmetic applications [137, 138]. More recently, xanthan has been used in the new clear-gel toothpastes.

Gellan has applications in the food industry as a gelling agent in frostings, glazes, icings, jam and jellies. *Sphingomonas* gellan (S-gellan), modified with diethylaminoethyl chloride-HCl (DEAE-HCl) is a polyelectrolyte that contains both positive and negative charges. The solubility of native S-gellan was improved, increased by nearly a factor of two, from 40 per cent to 75 per cent , after DEAE derivatization, while its water-holding and oil-binding capacities were drastically decreased. These improved properties and stronger bile acid binding capacity suggest that the DEAE-derivatized gellan has more advantages than gellan itself for functional food applications [139]. Films of gellan were implanted for insulin delivery in diabetic rats. It was found that their blood glucose levels were about half of those of rats implanted with blank films and that the therapeutic effect of insulin could last for 1 week. Both *in vitro* and *in vivo* studies indicated that the gellan film could be an ideal candidate for the development of protein delivery systems [140].

13.3 CONCLUDING REMARKS

The purpose of this chapter was to emphasize the interest of hemicelluloses as renewable resources capable of fulfilling a remarkable array of roles in today's science and technology. A better understanding of the physiological functions, chemistry and functionality of hemicelluloses constitutes a formidable task for the near future which should provide wider and more profound applications in areas such as materials science, medicine and biology.

REFERENCES

1. Ebringerova A., Heinze T., Xylan and xylan derivatives – Biopolymers with valuable properties, 1. Naturally occurring xylans structures, isolation procedures and properties, *Macromol. Rapid Comm.*, **21**(9), 2000, 542–556.
2. Viëtor R.J., Angelino S.A.G.F., Vosdragen A.G.J., Structural features of arabinoxylans from barley and malt cell wall material, *J. Cereal Sci.*, **15**, 1992, 213–222.
3. Carpita N.C., Structure and biogenesis of the cell walls grasses, *Annu. Rev. Plant Physiol. Plant Mol.*, **47**, 1996, 445–476.
4. Izydorczyk M.S., Biliaderis C.G., Cereal arabinoxylans: Advances in structure and physicochemical properties, *Carbohyd. Polym.*, **28**, 1995, 33–48.
5. Andrewartha K.A., Phillips D.R., Stone B.A., Solution properties of wheat-flour arabinoxylans and enzymically modified arabinoxylans, *Carbohyd. Res.*, **77**, 1979, 191–204.
6. Fincher G.B., Stone B.A., Cell walls and their components in cereal grain technology, in *Advances in Cereal Science and Technology*, Ed.: Pomeranz Y., American Association of Cereal Chemists, St. Paul, MN, 1986, **Vol. 8**, pp. 207–295.
7. Vinkx C.J.A., Delcour A., Rye (*Secale cereale* L.) arabinoxylans: A critical review, *J. Cereal Sci.*, **24**, 1996, 1–14.
8. Ferre H., Broberg A., Duus J.O., Thomsen K.K., A novel type of arabinoxylan arabinofuranosylhydrolase from germinated barley, *Eur. J. Biochem.*, **267**(22), 2000, 6633–6641.
9. Bauer W.D., Talmadge K.W., Keegstra K., Albersheim P., The structure of plant cell walls: II. The hemicellulose of the walls of suspension-cultured sycamore cells, *Plant Physiol.*, **51**(1), 1973, 174–187.
10. Kennedy J.F., Paterson M., Bioconversion of wood and cellulosic materials, in *Cellulosic Utilization [Proceedings of International Conference on cellulosics utilization in the new future]*, Eds.: Inagaki H. and Phillips G.O., Elsevier Applied Science, 1988, pp. 203–212.
11. Popa V.I., Enzymatic hydrolysis of hemicelluloses and cellulose, in *Polysaccharides. Structural Diversity and Functional Versatility*, Ed.: Dumitriu S., Marcel Dekker, 1998, pp. 969–1007.
12. Sjöström E., *Wood Chemistry. Fundamentals and Applications*, 2nd Edition, Academic Press, San Diego, CA, USA, 1993. p. 293.
13. Shimizu K., Chemistry of hemicelluloses, in *Wood and Cellulosic Chemistry,* Eds.: Hon D.N.-S., Shiraishi N., 2nd Edition, Marcel Dekker Inc., New York, USA, 2001, pp. 177–214.
14. Dea I.C.M., Morrison A., Chemistry and interactions of seed galactomannans, *Adv. Carbohyd. Chem. Biochem.*, **31**, 1975, 241–312.
15. Dey P.M., Biochemistry of plant galactomannans, *Adv. Carbohyd. Chem. Biochem.*, **35**, 1978, 341–376.
16. Chandrasekaran R., Janaswamy S., Morphology of western larch arabinogalactan, *Carbohyd. Res.*, **337**(21), 2002, 2211–2222.
17. Aspinall G.O., Chemistry of cell wall polysaccharides, in *The Biochemistry of Plants*, Ed.: Preiss J., Academic Press, London, 1980, **Vol. 3**, pp. 473–500.
18. Wilkie K.C.B., The hemicelluloses of grasses and cereals, *Adv. Carbohyd. Chem. Biochem.*, **36**, 1979, 215–264.
19. Dervilly-Pinel G., Tran V., Saulnier L., Investigation of the distribution of arabinose residues on the xylan backbone of water-soluble arabinoxylans from wheat flour, *Carbohyd. Polym.*, **55**(3), 2004, 171–177.
20. Smith M.M., Hartley R.D., Occurrence and nature of ferulic acid substitution of cell wall polysaccharides in graminaceous plants, *Carbohyd. Res.*, **118**, 1983, 65–80.
21. Sorensen H.R., Meyer A.S., Pedersen S., Enzymatic hydrolysis of water-soluble wheat arabinoxylan. 1. Synergy between alpha-L-arabinofuranosidases, endo-1, 4-beta-xylanases, and beta-xylosidase activities, *Biotechnol. Bioeng.*, **81**(6), 2003, 726–731.
22. Tuncer M., Ball A.S., Co-operative actions and degradation analysis of purified xylan-degrading enzymes from *Thermomonospora fusca* BD25 on oat-spelt xylan, *J. Appl. Microbiol.*, **94**(6), 2003, 1030–1036.
23. Chaplin M.F., The structure of *Plantago ovata* arabinoxylan, in *Gums and stabilisers for the food industry*, Eds.: Williams P.A., Phillips G.O., Royal Society of Chemistry, 2004, pp. 509–516.
24. Jarvis M.C., Briggs S.P.H., Knox J.P., Intercellular adhesion and cell separation in plant, *Plant Cell Environ.*, **26**(7), 2003, 977–989.
25. Brent L.R., Molcolm A.O., Debra M., Pectins: Structure, biosynthesis and oligosaccharide-related signaling, *Phytochemistry*, **57**, 2001, 929–967.
26. Vincker J.-P., Schols H.A., Oomen R.J.F.J., Beldman G., Visser R.G.F., Voragen A.G.J., Pectin – The hairy thing, in *Advances in Pectin and Pectinase Research,* Eds.: Voragen A.G.J., Schols H. and Visser R., Kluwer Academic Publishers, 2003, pp. 47–59.
27. Aspinall G.O., Baillie J., Gum tragacanth. I., Fractionation of the gum and structure of tragacanthic acid, *J. Chem. Soc.*, **318**, 1963, 1702–1714.
28. Grant G.T., Morris E.R., Rees D.A., Smith P.C.J., Thom D., Biological interaction between polysaccharides and divalent cations: The egg-box model, *FEBS Lett.*, **32**, 1963, 195–198.

29. Takano R., Shimoto K., Kamei K., Hara S., Hirase S., Occurrence of carrageenan structure in an agar from the red seaweed *Digenea simplex* (Wulfen) *C. Agardh* (Rhodomelaceae, Ceramiales) with a short review of carrageenan–agarocolloid hybrid in the *Florideophycidae*, *Bot. Mar.*, **46**(2), 2003, 142–150.
30. Tako M., Higa M., Medoruma K., Nakasone Y., A highly methylated agar from red seaweed, *Gracilaria arcuata*, *Bot. Mar.*, **42**(6), 1999, 513–517.
31. Mitsuiki M., Mizuno A., Motoki M., Determination of molecular weight of agars and effect of the molecular weight on the glass transition, *J. Agric. Food Chem.*, **47**(2), 1999, 473–478.
32. Lahaye M., Rochas C., Chemical structure and physico-chemical properties of agar, *Hydrobiologia*, **221**(1), 1991, 137–148.
33. Norton I.T., Goodall D.M., Austin K.R.J., Morris E.R., Rees D.A., Dynamics of molecular organization in agarose sulphate, *Biopolymers*, **25**(6), 1986, 1009–1029.
34. Arnott S., Fulmer A., Scott W.E., Dea I.C.M., Moorhouse R., Rees D.A., The agarose double helix and its function in agarose gel structure, *J. Mol. Biol.*, **90**(2), 1974, 269–284.
35. Haug A., Larsen B., Smidsrod O., Studies on the sequence of uronic acid residues in alginic acid, *Acta Chem. Scand.*, **21**, 1967, 691–704.
36. Wang, Z.-Y., Zhang Q.-Z., Konno M., Saito S., Sol–gel transition of alginate solution by addition of calcium ions: Alginate concentration dependence of gel point, *J. Phys. II France*, **3**, 1993, 1–7.
37. Kim Y.T., Kim E.H., Cheong C., Williams D.L., Kim C.W., Lim S.T., Structural characterization of β-(1 → 3, 1 → 6)-linked glucans using NMR spectroscopy, *Carbohyd. Res.*, **328**(3), 2000, 331–341.
38. Harada T., Amemura A., Saito H., Kanamaru S., Misaki A., Formation of succinoglucan and curdlan by parent and mutant strains of *Alcaligenes faecalis* var. *myxogenes* 10C3, *J. Ferment. Technol.*, **46**, 1968, 679–684.
39. Saudagar P.S., Singhal R.S., Fermentative production of curdlan, *Appl. Biochem. Biotechnol.*, **118**(1–3), 2004, 21–31.
40. McIntosh M., Stone B.A., Stanisich V.A., Curdlan and other bacterial (1 → 3)-β-D-glucans, *Appl. Microbiol. Biotechnol.*, **68**(2), 2005, 163–173.
41. Chan T.W., Tang K.Y., Analysis of a bioactive β-(1 → 3) polysaccharide (curdlan) using matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry, *Rapid Commun. Mass Spectrom.*, **17**(9), 2003, 887–896.
42. Okuyama K., Otsubo A., Fukuzawa Y., Ozawa M., Harada T., Kasai N., Single-helical structure of native curdlan and its aggregation state, *J. Carbohyd. Chem.*, **10**(4), 1991, 645–656.
43. Zhang H., Nishinari K., Williams M.A., Foster T.J., Norton I.T., A molecular description of the gelation mechanism of curdlan, *Int. J. Biol. Macromol.*, **30**(1), 2002, 7–16.
44. Ikeda S., Shishido Y., Atomic force microscopy studies on heat-induced gelation of curdlan, *J. Agric. Food Chem.*, **53**(3), 2005, 786–791.
45. Zhang H., Huang L., Nishinari K., Watase M., Konno A., Thermal measurements of curdlan in aqueous suspension during gelation, *Food Hydrocolloid.*, **14**(2), 2000, 121–124.
46. Palleschi A., Bocchinfuso G., Coviello T., Alhaique F., Molecular dynamics investigations of the polysaccharide scleroglucan: First study on the triple helix structure, *Carbohyd. Res.*, **340**(13), 2005, 2154–2162.
47. Deslandes Y., Marchessault R.H., Sarko A., Triple-helical structure of (1,3)-β-D-glucan, *Macromolecules*, **13**, 1980, 1466–1471.
48. Blum T.L., Deslandes Y., Marchessault R.H., Perez S., Rinaudo M., Solid-state and solution conformations of scleroglucan, *Carbohyd. Res.*, **100**, 1982, 117–130.
49. Yanaki T., Norisuye T., Triple helix and random coil scleroglucan in dilute solution, *Polymer J.*, **15**(5), 1983, 389–396.
50. Leathers T.D., Biotechnological production and applications of pullulan, *Appl. Microbiol. Biotechnol.*, **62**(5–6), 2003, 468–473.
51. Auer D.P.F., Seviour R.J., Influence of varying nitrogen sources on polysaccharide production by *Aureobasidium pullulans* in batch culture, *Appl. Microbiol. Biotechnol.*, **32**, 1990, 637–644.
52. Badr-Eldin S.M., El-Tayeb O.M., El-Masry E.G., Mohamad O.A., El-Rahman O.A.A., Polysaccharide production by *Aureobasidium pullulans*: Factors affecting polysaccharide formation, *World J. Microbiol. Biotechnol.*, **10**(4), 1994, 423–426.
53. Wiley B.J., Ball D.H., Arcidiacono S.M., Mayer J.M., Kaplan D.L., Control of molecular weight distribution of the biopolymer pullulan produced by *Aureobasidium pullulans*, *J. Environ. Polym. Degrad.*, **1**, 1993, 3–9.
54. Roukas T., Biliaderis C.G., Evaluation of carob pod as a substrate for pullulan production by *Aureobasidium pullulans*, *Appl. Biochem. Biotechnol.*, **55**(1), 1995, 27–44.
55. Kato I., Okamoto T., Tokuya T., Takahashi A., Solution properties and chain flexibility of pullulan in aqueous solution, *Biopolymers*, **21**, 1982, 1623–1633.
56. Alasdair F.B., Lutz H., Laurence D.B., Polysaccharides vibrational Raman optical activity: Laminarin and pullulan, *J. Raman Spectrosc.*, **26**(12), 1995, 1071–1074.
57. Lee J.W., Yeomans W.G., Allen A.L., Deng F., Gross R.A., Kaplan D.L., Biosynthesis of novel exopolymers by *Aureobasidium pullulans*, *Appl. Environ. Microbiol.*, **65**(12), 1999, 5265–5271.
58. Behravan J., Bazzaz B.S., Salimi Z., Optimization of dextran production by *Leuconostoc mesenteroides* NRRL B-512 using cheap and local sources of carbohydrates and nitrogen, *Biotechnol. Appl. Biochem.*, **38**(3), 2003, 267–269.

59. Chicoine L.M., Suppiramaniam V., Vaithianathan T., Gianutsos G., Bahr B.A., Sulfate- and size-dependent polysaccharide modulation of AMPA receptor properties, *J. Neurosci. Res.*, **75**(3), 2004, 408–416.
60. Antonini E., Bellelli L., Bruzzesi M.R., Caputo A., Chiancone E., Rossi-Fanelli A., Studies on dextran and dextran derivatives. I. Properties of native dextran in different solvents, *Biopolymers*, **2**(1), 2004, 27–34.
61. Jansson P.E., Kenne L., Lindberg B., Structure of the extracellular polysccharide from *Xanthomonas campestris*, *Carbohyd. Res.*, **45**, 1975, 275–282.
62. Papagianni M., Psomas S.K., Batsilas L., Paras S.V., Kyriakidis D.A., Liakopoulou-Kyriakides M., Xanthan production by *Xanthomonas campestris* in batch cultures, *Process Biochem.*, **37**(1), 2001, 73–80.
63. Kennedy J.F., Jones P., Barker S.A., Factors affecting microbial growth and polysaccharide production during the fermentation of *Xanthomonas campestris* culture, *Enz. Microb. Technol.*, **4**, 1982, 39–43.
64. Becker A., Katzen F., Pühler A., Ielpi L., Xanthan biosynthesis and function: A biochemical-genetic perspective, *Appl. Microbiol. Biotechnol.*, **50**(2), 1998, 145–152.
65. Ielpi L., Couso R.O., Dankert M.A., Sequential assembly and polymerization of the polyprenol-linked pentasaccharide repeating unit of the xanthan polysaccharide in *Xanthomonas campestris*, *J. Bacteriol.*, **175**(9), 1993, 2490–2500.
66. Mila M., Rinaudo M., Tinland B., de Murcia G., Evidence for a single stranded xanthan by electron microscopy, *Polym. Bull*, **19**, 1988, 567–572.
67. Moorhouse R., Colegrove G.T., Sandford P.A., Baird J.K., Kang K.S., PS-60: A new gel-forming polysaccharide, *Am. Chem. Soc. Symp. Ser.*, **150**, 1981, 111–124.
68. Rinaudo M., Milas M., Xanthan properties in aqueous solution, *Carbohyd. Polym.*, **2**(4), 1982, 264–269.
69. Sanderson G.R., Gellan gum, in *Food Gels,* Ed.: Harries P., Elsevier Science Publishing, New York, 1990, pp. 201–233.
70. Kuo M.S., Dell A., Mort A.J., Identification and location of L-glycerate, unusual acyl substitution in gellan gum, *Carbohyd. Res.*, **156**, 1986, 173–187.
71. Fialho A.M., Martins L.O., Donval M.L., Leitão J.H., Ridout M.J., Jay A.J., Morris V.J., Correia I.Sá., Structures and properties of gellan polymers produced by *Sphingomonas paucimobilis* ATCC 31461 from lactose compared with those produced from glucose and from cheese whey, *Appl. Environ. Microbiol.*, **65**(6), 1999, 2485–2491.
72. Chandrasekaran R., Radha A., Thailambal V.G., Roles of potassium ions, acetyl and L-glyceryl groups in native gellan double helix: An X-ray study, *Carbohyd. Res.*, **224**, 1992, 1–17.
73. Groman E.V., Enriquez P.M., Jung C., Josephson L., Arabinogalactan for hepatic drug delivery, *Bioconjugate Chem.*, **5**(6), 1994, 547–556.
74. Gregory S., Kelly N.D., Larch arabinogalactan. Clinical relevance of a novel immune-enhancing polysaccharide, *Altern. Med. Rev.*, **4**(2), 1999, 96–103.
75. Lu Z.X., Walker K.Z., Muir J.G., O'Dea K., Arabinoxylan fibre improves metabolic control in people with Type II diabetes, *Eur. J. Clin. Nutr.*, **58**(4), 2004, 621–628.
76. Govers M.J., Gannon M.J., Dunshea F.R., Gibson P.R., Muir J.G., Wheat bran affects the site of fermentation of resistant starch and luminal indexes related to colon cancer risk: A study in pigs, *Gut*, **45**(6), 1999, 840–847.
77. Grodhal M., Eriksson L., Gatenholm P., Material properties of plasticized hardwood xylans for potential application as oxygen barrier films, *Biomacromolecules*, **5**(4), 2004, 1528–1535.
78. Fukunaga T., Sasaki M., Araki Y., Okamoto T., Yasuoka T., Tsujikawa T., Fujiyama Y., Bamba T., Effects of the soluble fibre pectin on intestinal cell proliferation, fecal short chain fatty acid production and microbial population, *Digestion*, **67**(1–2), 2003, 42–49.
79. Yamaguchi F., Uchida S., Watabe S., Kojima H., Shimizu N., Hatanaka C., Relationship between molecular weights of pectin and hypocholesterolemic effects in rats, *Biosci. Biotechnol. Biochem.*, **59**(11), 1995, 2130–2131.
80. Judd P.A., Truswell A.S., The hypocholesterolemic effects of pectins in rats, *Br. J. Nutr.*, **53**(3), 1985, 409–425.
81. Anderson J.W., Hanna T.J., Impact of nondigestible carbohydrates on serum lipoproteins and risk for cardiovascular disease, *J. Nutr.*, **129**(7), 1999, 1457–1466.
82. Chiellini F., Perspectives on *in vitro* evaluation of biomedical polymers, *J. Bioact. Compat. Pol.*, **21**(3), 2006, 257–271.
83. Paper D.H., Vogl H., Franz G., Hoffman R., Defined carrageenan derivatives as angiogenesis inhibitors, *Macromol. Symp.*, **99**, 1995, 219–225.
84. Lai L.S., Lin P.H., Application of decolourised Hsian-tsao leaf gum to low-fat salad dressing model emulsions: A rheological study, *J. Sci. Food Agric.*, **84**(11), 2004, 1307–1314.
85. Jackson G., Roberts R.T., Wainwright T., Mechanism of beer foam stabilization by propylene glycol alginate, *J. Inst. Brew.*, **86**, 1980, 34–37.
86. O'Reilly J., The role of enhanced solubility PGA in beer head retention, *Brew. Guard.*, **125**(7), 1996, 22–24.
87. Orive G., Tam S.K., Pedraz J.L., Halle J.P., Biocompatibility of alginate-poly-L-lysine microcapsules for cell therapy, *Biomaterials*, **27**(20), 2006, 3691–3700.
88. Dufrane D., Steenberghe M., Goebbels R.M., Saliez A., Guiot Y., Gianello P., The influence of implantation site on the biocompatibility and survival of alginate encapsulated pig islets in rats, *Biomaterials*, **27**(17), 2006, 3201–3208.

89. Liew C.V., Chan L.W., Ching A.L., Heng P.W., Evaluation of sodium alginate as drug release modifier in matrix tablets, *Int. J. Pharm.*, **309**(1–2), 2006, 25–37.
90. Thanos C.G., Bintz B.E., Bell W.J., Qian H., Schneider P.A., MacArthur D.H., Emerich D.F., Intraperitoneal stability of alginate-polyornithine microcapsules in rats: An FTIR and SEM analysis, *Biomaterials*, **27**(19), 2006, 3570–3579.
91. Sankalia M.G., Mashru R.C., Sankalia J.M., Sutariya V.B., Papain entrapment in alginate beads for stability improvements and site-specifica delivery: Physicochemical chracaterisation and factorial optimization using neural network modeling, *AAPS Pharm. Sci. Tech.*, **6**(2), 2005, 209–222.
92. Nagakura T., Hirata H., Tsujii M., Sugimoto T., Miyamoto K., Horiuchi T., Nagao M., Nakashima T., Uchida A., Effect of viscous injectable pure alginate sol on cultured fibroblasts, *Plast. Reconstr. Surg.*, **116**(3), 2005, 831–838.
93. Rowley J.A., Madlambayan G., Mooney D.J., Alginate hydrogels as synthetic extracellular materials, *Biomaterials*, **20**(1), 1999, 45–53.
94. Suzuki Y., Tanihara M., Suzuki K., Saitou A., Sufan W., Nishimura Y., Alginate, hydrogel linked with synthetic oligopeptide derived from BMP-2 allows ectopic osteoinduction *in vivo*, *J. Biomed. Mater. Res.*, **50**(3), 2000, 405–409.
95. Atala A., Kim W., Paige K.T., Vacanti C.A., Retik A.B., Endoscopic treatment of vesicoureteral reflux with a chondrocyte-alginate suspension, *J. Urol.*, **152**(2), 1994, 641–643.
96. Elcin Y.M., Dixit V., Gitnick T., Extensive *in vivo* angiogenesis following controlled release of human vascular endothelial cell growth factor: Implications for tissue engineering and wound healing, *Artif. Organs.*, **25**(7), 2001, 558–565.
97. Soon-Shiong P., Otterlie M., Skjak-Bræek S., Smidsrød O., Heintz R., Lanza R.P., Espevik T., An immunologic basis for the fibrotic reaction to implanted micro-capsules, *Transplant. Proc.*, **23**(1), 1991, 758–759.
98. Suzuki K., Suzuki Y., Tanihara M., Ohnishi K., Hashimoto T., Endo K., Nishimura Y.Y., Reconstruction of rat peripheral nerve gap without sutures using freeze-dried alginate gel, *J. Biomed. Mater. Res.*, **49**(4), 1999, 528–533.
99. Edwards P.C., Mason G.M., Gene-enhanced tissue engineering for dental hard tissue regeneration: (1) Overview and practical considerations, *Head Face Med.*, **2**, 2006, 12–16.
100. Zülli F., Suter F., Biltz H., Nissen H.P., Birman M., Carboxymethylated β(1,3)-glucan, a beta glucan from baker's yeast helps protect skin, *Cosmet. Toiletries*, **111**(12), 1996, 91–98.
101. Ohno N., Furukawa M., Miura N.N., Adachi Y., Motol M., Yadoma T., Antitumor β-glucan from the cultured fruit body of *Agaricus blazei*, *Biol. Pharm. Bull.*, **24**(7), 2001, 820–828.
102. Camelini C.M., Maraschin M., de Mendoca M.M., Zucco C., Ferreira A.G., Tavares L.A., Structural characterization of beta-glucans of *Agaricus brasiliensis* in different stages of fruiting body maturity and their use in nutraceutical products, *Biotechnol. Lett.*, **27**(17), 2005, 1295–1299.
103. Kaneno R., Fontanari L.M., Santos S.A., Di Stasi L.C., Rodrigues Filho E., Eira A.F., Effects of extracts from Brazilian sun-mushroom (*Agaricus blazei*) on the NK activity and lymphoproliferative responsiveness of Ehrlich tumor-bearing mice, *Food Chem. Toxicol.*, **42**(6), 2004, 909–916.
104. Di Luzio N.R., Immunopharmacology of glucan: A broad spectrum enhancer of host defense mechanisms, *Trends Pharmacol. Sci.*, **4**(8), 1983, 344–348.
105. Sakurai T., Ohno N., Yadomae T., Changes in immune mediators in mouse lung produced by administration of soluble (1,3)-beta-D-glucan, *Biol. Pharm. Bull.*, **17**(5), 1994, 617–622.
106. Ha C.H., Lim K.H., Jang S.H., Yun C.W., Paik H.D., Kim S.W., Kang C.W., Chang H.I., Immune-enhancing alkali-soluble glucans produced by wild-type and mutant *Saccharomyces cerevisiae*, *J. Microbiol. Biotechnol.*, **16**(4), 2006, 576–583.
107. Nakao Y., Konno A., Taguchi T., Tawada T., Kasai H., Toda J., Terasaki M., Curdlan: Properties and applications to foods, *J. Food Sci.*, **56**, 1991, 769–776.
108. Nakao Y., Suzuki K., Curdlan: Properties and applications to foods, *Int. Food Ingredients*, **5**, 1994.
109. Funami T., Yada H., Nakao Y., Curdlan properties for application in fat mimetics for meat products, *J. Food Sci.*, **63**(22), 1998, 283–287.
110. Sasaki T., Tanaka M., Uchida H., Effect of serum from mice treated with antitumor polysaccharides on expression of cytotoxicity by mouse peritoneal macrophages, *J. Pharmacobio-dynam.*, **5**(12), 1982, 1012–1016.
111. Morikawa K., Takeda S., Yamazaki M., Mizuno D., Induction of tumoricid activity of polymorphonuclear leukocytes by a linear β-1,3-D-glucan and other immunomodulators in murine cells, *Cancer Res.*, **45**(4), 1985, 1496–1501.
112. Aoki T., Kaneko Y., Stefanski M.S., Nguyen T., Ting R.C., Curdlan sulfate and HIV-1: *In vitro* inhibitory effects of curdlan sulfate on HIV-1 infection, *AIDS Res. Hum. Retrov.*, **7**(4), 1991, 409–415.
113. Osawa Z., Morota T., Hatanaka K., Akaike T., Matsuzaki K., Nakashima H., Yamamoto N., Suzuki E., Miyano H., Mimura T., Kaneko Y., Synthesis of sulfated derivatives of curdlan and their antiHIV activity, *Carbohyd. Polym.*, **21**, 1993, 283–288.
114. Miao H.Q., Ishai-Michaeli R., Peretz T., Vlodavsky I., Laminarin sulfate mimics the effects of heparin on smooth muscle cell proliferation and basic fibroblast growth factor-receptor binding and mitogenic activity, *J. Cell Physiol.*, **164**(3), 1995, 482–490.
115. Pang Z., Otaka K., Maoka T., Hidaka K., Ishijima S., Oda M., Ohnishi M., Structure of beta-glucan oligomer from laminarin and its effect on human monocytes to inhibit the proliferation of receptor binding in the CR3- human promonocytic cell line U937, *Biosci. Biotechnol. Biochem.*, **69**(3), 2005, 553–558.

116. Cho C.C.M., Liu E.S.L., Shin V.Y., Cho C.H., Polysaccharides: A new role in gastrointestinal protection, in *Gastrointestinal Mucosal Repair and Experimental Therapeutics*. Eds.: Cho C.-H., Wang J.-Y., Front. Gastrointest. Res., Basel, Karger, 2002, **Vol. 25**, p. 180–189.
117. Aziz A., Poinssot B., Daire X., Adrian M., Bezier A., Lambert B., Joubert J.M., Pugin A., Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea and Plasmopara viticola*, *Mol. Plant Microbe. In.*, **16**(12), 2003, 1118–1128.
118. Hoffman R., Paper D.H., Donaldson J., Vogl H., Inhibition of angiogenesis and murine tumour growth by laminarin sulphate, *Br. J. Cancer*, **73**(10), 1996, 1183–1185.
119. Coviello T., Palleschi A., Grassi M., Matricardi P., Bocchinfuso G., Alhaique F., Scleroglucan: A versatile polysaccharide for modified drug delivery, *Molecules*, **10**(1), 2005, 6–33.
120. Bousquet M., Escoula L., Peuriere S., Pipy B., Roubinet F., Chavant C., Immunopharmacologic study in mice of 2 β-1,3, beta-1, 6 polysaccharides (scleroglucan and PSAT) on the activation of macrophages and T lymphocytes, *Ann. Rech. Vet.*, **20**(2), 1989, 165–173.
121. Pretus H.A., Ensley H.E., McNamee R.B., Jones E.L., Browder I.W., Williams D.L., Isolation, physicochemical characterization and preclinical efficiency evaluation of soluble scleroglucan, *J. Pharmacol. Exp. Ther.*, **257**(1), 1991, 500–510.
122. Matsuyama H., Mangindaan R.E.P., Yano T., Protective effect of schizophyllan and scleroglucan against *Streptococcus* sp. infection in yellowtail (*Seriola quinqueradiata*), *Aquaculture*, **101**, 1992, 197–203.
123. Sallustio S., Galantini L., Gente G., Masci G., La Mesa C., Hydrophobically modified pullulans: Characterization and physicochemical properties, *J. Phys. Chem. B*, **108**(49), 2004, 18876–18883.
124. Reza M., Recent trends in the use of polysaccharides for improved delivery of therapeutic agents: Pharmacokinetic and pharmacodynamic perspectives, *Curr. Pharm. Biotechnol.*, **4**(5), 2003, 283–302.
125. Alban S., Schauerte A., Franz G., Anticoagulant sulfated polysaccharides: Part I. Synthesis and structure–activity relationships of new pullulan sulfates, *Carbohyd. Polym.*, **47**, 2002, 267–276.
126. Kandemir N., Yemenicioglu A., Mecitoglu Ç., Elmaci Z.S., Arslanogwlu A., Göksungur Y., Baysal T., Production of antimicrobial films by incorporation of partially purified lysozyme into biodegradable films of crude exopolysaccharides obtained from *Aureobasidium pullulans* fermentation, *Food Technol. Biotechnol.*, **43**(4), 2005, 343–350.
127. Uchida T., Ikegami H., Ando S., Kurimoto M., Mitsuhashi M., Naito S., Usui M., Matuhasi T., Suppression of murine IgE response with ovalbumin–pullulan conjugates: Comparison of the suppressive effect of different conjugation methods and different molecular weights of pullulan, *Int. Arch. Allergy Immunol.*, **102**(3), 1993, 276–278.
128. Ferreira L., Gil M.H., Cabrita A.M., Dordich J.S., Biocatalytic synthesis of highly ordered degradable dextran-based hydrogels, *Biomaterials*, **26**(23), 2005, 4707–4716.
129. Cascone M.G., Polacco G., Lazzeri L., Barbani N., Dextran/poly(acrylic acid) mixtures as miscible blends, *J. Appl. Polym. Sci.*, **66**(11), 1997, 2089–2094.
130. Ciardelli G., Chiono V., Vozzi G., Pracella M., Ahluwalia A., Barbani N., Cristallini C., Giusti P., Blends of poly-ε-(caprolactone) and polysaccharides in tissue engineering applications, *Biomacromolecules*, **6**(4), 2005, 1961–1976.
131. Lundblad R., Bradshaw R.A., Applications of site-specific chemical modification in the manufacture of biopharmaceuticals: I. An overview, *Biotechnol. Appl. Biochem.*, **26**(3), 1997, 143–151.
132. Kaplan J.M., Pennington S.E., George J.A., Woodworth L.A., Fasbender A., Marshall J., Cheng S.H., Wadsworth S.C., Gregory R.J., Smith A.E., Potentiation of gene transfer to the mouse lung by complexes of adenovirus vector and polycations improves therapeutic potential, *Hum. Gene Ther.*, **9**(10), 1998, 1469–1479.
133. Liptay S., Weidenbach H., Adler G., Schmid R.M., Colon epithelium can be transiently transfected with liposomes, calcium phosphate precipitation and DEAE dextran *in vivo*, *Digestion*, **59**(2), 1998, 142–147.
134. Flexner C., Barditch-Crovo P.A., Kornhauser D.M., Farzadegan H., Nerhood L.J., Chaisson R.E., Bell B.E., Lorentsen M.J., Hendrix C.W., Petty B.G., Pharmacokinetics, toxicity, and activity of intravenous dextran sulfate in human immunodeficiency virus infection, *Antimicrob. Agents Chemother.*, **35**(12), 1991, 2544–2550.
135. Dyer A.P., Banfield B.W., Martindale B., Spannier D.M., Tufaro E., Dextran sulfate can act as an artificial receptor to mediate a type-specific herpes simplex virus infection via glycoprotein B, *J. Virol.*, **71**(1), 1997, 191–198.
136. Krumbiegel M., Dimitrov D.S., Puri A., Blumenthal R., Dextran sulfate inhibits fusion of *influenza* virus and cells expressing influenza hemagglutinin with red blood cells, *Biochim. Biophys. Acta*, **1110**(2), 1992, 158–164.
137. Ruissen L.A., Vander Reijden W.A., van't Hof W., Veerman E.C.I., Nieuw Amerongen A.V., Evaluation of the use of xanthan as vehicle for cationic antifungal peptides, *J. Control Release*, **60**(1), 1999, 49–56.
138. Brookshier K.A., Tarbell J.M., Evaluation of a transparent blood analog fluid: Aqueous xanthan gum/glycerin, *Biorheology*, **30**(2), 1993, 107–116.
139. Yoo S-H., Kyung H.L., Lee J.S., Cha J., Cheon S.P., Hyeon G.L., Physicochemical properties and biological activities of DEAE-derivatized *Sphingomonas* gellan, *J. Agric. Food Chem.*, **53**(16), 2005, 6235–6239.
140. Li J., Kamath K., Dwivedi C., Gellan film as an implant for insulin delivery, *J. Biomater. Appl.*, **15**(4), 2001, 321–343.

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Cork and Suberins: Major Sources, Properties and Applications

Armando J.D. Silvestre, Carlos Pascoal Neto and Alessandro Gandini

ABSTRACT

The main purpose of this chapter is to discuss the potential of suberin (an aromatic–aliphatic crosslinked polyester widespread in the plant kingdom) as a renewable source of chemicals and, in particular, of macromonomers. Despite being widespread in plants, only two species produce suberin-rich biomass residues in amounts that justify their exploitation as renewable sources of chemicals and monomers, namely *Quercus suber* with cork and *Betula pendula* (birch) with its outer bark. *Quercus suber* cork is a material with unique properties and applications, whereas to the best of our knowledge, the outer bark of birch finds no direct applications. Hence, this chapter first provides a general overview of the properties and applications of cork, as well as of its utilization as a starting material for the synthesis of liquid polyols, before dealing with the macromolecular structure of suberin (the major cork component), its depolymerization methods, and the composition and applications of the ensuing fragment mixtures.

Keywords

Quercus suber, Betula pendula, Cork, Suberin, Chemical composition, Aliphatic macromonomers, Polyurethanes, Polyesters

14.1 INTRODUCTION

The present chapter aims essentially at presenting the potentials of suberin as a renewable source of chemicals and more specifically of macromonomers. Suberin is a naturally occurring aromatic–aliphatic crosslinked polyester widespread in the plant kingdom, where it plays a key role as a protective barrier between the plant and the surrounding environment. It is found mainly in the cell walls of normal and wounded external tissues of aerial and/or subterranean parts of many plants, mainly in the outer bark of higher plants and in tuber periderms [1].

Despite being widespread in plants [1], only two species produce suberin-rich biomass residues in amounts that justify their exploitation as renewable sources of chemicals for polymer synthesis, namely *Quercus suber* with cork and *Betula pendula* (birch) with its outer bark. *Quercus suber* cork is a material with unique properties and applications, whereas to the best of our knowledge, the outer bark of birch finds no direct applications.

The present chapter will, therefore, first give a general overview of the properties and applications of cork, as well as of its utilization as a starting material for the synthesis of liquid polyols, before dealing with the macromolecular structure of suberin, its depolymerization methods, and the composition and applications of the ensuing fragment mixtures.

14.2 CORK

Cork is the common designation of the suberized bark of cork oak (*Quercus suber* L.), an evergreen tree that grows in the western Mediterranean region [2, 3]. Although other species contain suberized tissues, no other tree in the world gives rise to the thick layers of suberized bark (cork) generated by *Quercus suber*. Cork is therefore a unique suberized tissue and an important non-wood forest product which finds a wide domain of utilizations, such as wine and champaign stoppers, insulating materials, marine floats and household and office indoor panels, some of which date back to Egyptian, Greek and Roman times [3].

Among the countries involved in the cultivation of *Quercus suber* and the production of cork, Portugal is largely the leader with an annual production of 185 000 ton, which constitutes about 50 per cent of the world output [4]. Although *Quercus suber* is a protected species and a key element in the southern Portugal ecosystem and landscape, cork exploitation is a fully sustainable activity, if adequately carried out by professionals. Indeed, cork stripping which is carried out once every 9–12 years seems to have a positive effect on the tree's health and growth.

Cork is composed of suberin, the main component, lignin, polysaccharides and extractives, including mostly aliphatic, phenolic and triterpenenic compounds [2, 5–13]. The relative abundance of these elements is extremely variable, being influenced by the geographical origin and quality of the tree, and/or even the different parts of the tree from which the cork is harvested [5, 11, 12]. Suberin represents 38–62 per cent of the cork weight [14] and is by far the most original macromolecule present. The suberized cells, assembled in cork's peculiar hollow structure (Fig. 14.1), are responsible for the unique properties of this natural material [2, 3, 15–24], such as its remarkable elasticity, low density, impermeability to liquids and gases, low heat and sound conductivity, thermal and combustion resistance and resistance to rotting.

Apart from the major traditional cork applications mentioned above, these unique properties justify the fact that a wide variety of *high-tech* materials have been developed and are being sought in such diverse areas as industrial, household and sport [25] insulation, sealing and anti-vibration and even in the NASA Space Shuttle [26, 27]. Further applications are being developed for commercial jet liners [28]. Another interesting recent development related to a new family of composite materials, in which cork plays a key role, relates to its inclusion in cementitious materials in order to reduce their density [29].

In addition to both traditional and new applications of cork as a whole, whether used in its pristine form or after specific physical or chemical modifications, it is particularly relevant to emphasize that the interest of cork must also lie in the exploitation of some of its components, notably suberin extracted from cork processing by-products. Indeed, industrial cork processing generates substantial amounts of residues, such as the so-called *cork powder*, *black condensates* and *cooking waste waters*. Cork powder is generated during the production of granulated cork for agglomerated materials. Cork residues rejected during cork stopper production, are one of the main sources of granulated cork materials. The *cork powder* particles have an inadequate size distribution for their use in the manufacture of agglomerates and, at present, are mainly burned to produce energy [30, 31]. This by-product amounts, in Portugal, to about 40 000 ton per year (*i.e.* some 22 per cent of the national cork production). *Black condensates* are a residue of the production of black agglomerates, which involves the treatment of cork particles without any adhesive, at temperatures in the range of 250–500°C. During such thermal treatment, vapours are generated and

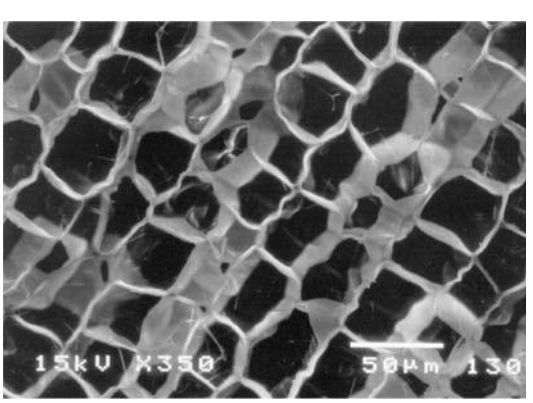

Figure 14.1 Electron microscopy of cork cell. (Reprinted with permission, Copyright 2002 by APCOR.)

later condensed in autoclave pipes. Periodically, this by-product is removed (2 500 ton per year in Portugal) and again burned to produce energy [30, 31]. Finally, *cooking waste waters* are the liquid effluents released during the treatment of cork planks with boiling water, a key stage in wine stopper production. The composition of this effluent is still poorly understood, however, it is known to be rich in phenolic compounds and not to contain suberin components; therefore its exploitation is out of the scope of this chapter.

Huge amounts of cork-based by-products are therefore potentially available for more noble uses than burning and hence they must be considered as particularly attractive renewable sources of chemical commodities, which fall neatly within the scope of the biorefinery concept in forest-based industries, which calls upon the upgrading of all the by-products [32].

Suberin is the privileged by-product in the context of this chapter, although its exploitation could well be integrated with the separation and use of high added-value low molecular weight components, such as friedeline, cerine, betulin and betulinic acid [33].

Given the specific purpose of this book, the present chapter will only deal with the macromolecular materials that can be obtained from cork and suberin and disregard all aspects related to the exploitation of other cork components.

14.2.1 The oxypropylation of cork

The oxypropylation of natural polymers has been applied successfully to a host of OH-bearing macromolecules, like cellulose, starch, chitin, chitosan, lignin and more complex agricultural by-products, such as sugar-beet pulp and olive stone powder, as discussed in detail in Chapter 12. In all instances, a nucleophilic catalyst (strong Brønsted bases like KOH work best) is used to deprotonate some of the substrate's OH groups and thus generate oxianions, which initiate the anionic polymerization of propylene oxide (PO), thereby inserting polyPO grafts onto the starting macromolecules. This reaction typically transforms the solid powder of the natural polymeric material into a viscous liquid polyol bearing as many OH groups as the initial substrate, since the oxypropylation is simply a 'chain extension' process. This branching mechanism is always accompanied by some PO homopolymerization, which produces oligomeric diols. Figure 14.2 provides a schematic view of the process, which requires typically temperatures above 130°C and PO pressures above 10 bar.

Cork powder was oxypropylated under these conditions [34,35] and the ensuing polyols fully characterized in terms of structure, homopolymer content, solubility, OH index and viscosity. The latter two parameters proved to be entirely comparable with those of commercial counterparts used in the manufacture of polyurethane materials. A study was therefore conducted [36] on the reactivity of the polyol mixtures, as obtained from the oxypropylation process, towards various diisocyanates and on the structure and properties of the ensuing polyurethanes.

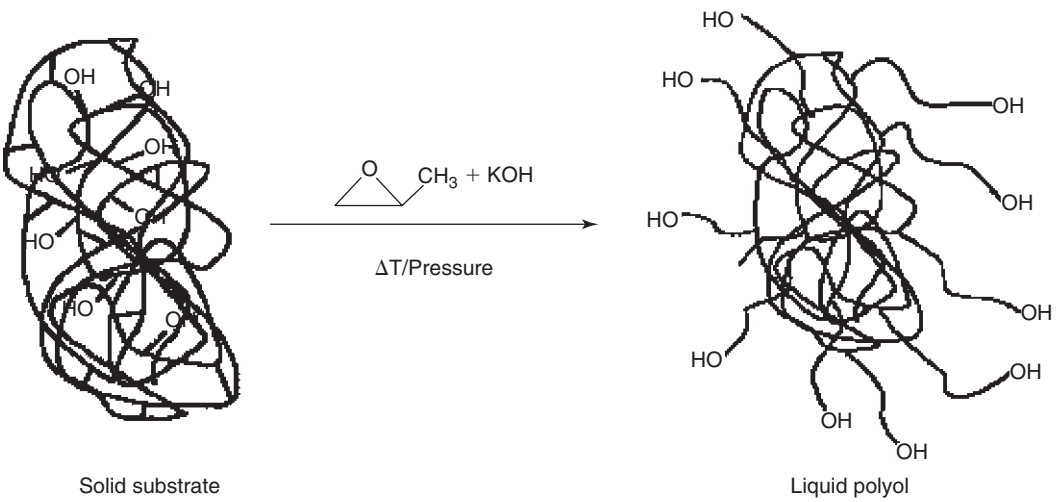

Figure 14.2 Schematic view of the oxypropylation of OH-bearing macromolecular materials.

This ongoing investigation is a good example of the interest in exploiting renewable resources, as emphasized in the broader context of Chapter 1, and the same strategy could equally be applied to other suberin-rich tree barks, such as that of birch.

14.3 SUBERIN

As mentioned above, and despite the fact that suberin is widespread in plants [1], only two species produce suberin-rich biomass residues in amounts that justify the exploitation of this natural polyester as a renewable source of chemicals for polymer synthesis, namely, *Quercus suber* and *Betula pendula. Betula pendula* is one of the most important industrial hardwood species in Northern Europe, where it is mainly used as raw material in the pulp and paper industry, which generates considerable amounts of bark. Typically, a mill with a pulp production of 400 000 ton per year, leaves about 28 000 ton per year of outer bark [37]. Considering a suberin content ranging from 32 to 59 per cent, [14], birch's outer bark has, like cork, an enormous potential as a source of suberin and suberin components.

Suberin contents ranging from 12 to 60 per cent have been reported for several other higher plant species namely *Acer griseum, Acer pseudoplatanus, Castanea sativa, Cupressus leylandii, Euonymus alatus, Fagus sylvatica, Fraxinus excelsior, Laburnum anagyroides, Populus tremula, Quercus ilex, Quercus robur, Ribes nigrum, Sambucus nigra* [38], *Pseudotuga menziesii* [39]) and for potato (*Solanum tuberosum*) periderm [40]. However, to the best of our knowledge, there is no industrial exploitation of these species in terms of suberin valorization. The only foreseeable exception could be, in the future, the exploitation of potato periderm as a by-product of the agro-food industry.

The interest in suberin lies in the fact that it constitutes an abundant source of ω-hydroxyfatty acids, α,ω-dicarboxylic acids and homologous mid-chain dihydroxy or epoxy derivatives [14]. Apart from this source, these compounds are not very abundant in nature. Thus, hydroxyfatty acids can only be additionally found in exploitable amounts in the seed oils of *Ricinus communis* (castor oil) and *Lesquerella spp.*, as well as in the extracellular aliphatic polyester covering most of the aerial surfaces of plants, known as cutin [41].

14.3.1 Suberin native structure

The suberin polymeric structure cannot be defined in terms of a monomer repeating unit, since the spatial arrangement of these moieties cannot be accurately defined, even when their relative abundance is known. Additionally, the latter aspect depends on the depolymerization methods used to isolate the aliphatic fraction and, moreover, as far as the aromatic domain is concerned, its identification/quantification is also quite complex because of its macromolecular nature and structural similarity with lignin [1, 42–45]. Notwithstanding these difficulties, several models have been proposed to illustrate the suberin macromolecular structure in suberized cell walls [46, 47]. In 2002, Bernards proposed a model for suberin from potato periderm, which summarized the state of the art on the structural data related to this macromolecular component [1].

The aliphatic domain of suberin (Fig. 14.3) is composed of branched polyester moieties mainly made up of long-chain ω-hydroxyfatty acids and α,ω-dicarboxylic acids, brought together through glycerol units [1, 48]. Glycerol has long been detected in suberin depolymerization extracts [43–45], but its role as a key structural building block was only demonstrated in more recent investigations [1, 39, 40, 49–52].

Solid-state NMR studies of cork [7, 53] and potato cell wall components [54–56], together with chemical analysis results, suggest the presence of two distinct aromatic domains in suberized cell walls (Fig. 14.3). The first, embedded in the aliphatic domains, is mainly composed of hydroxycinnamic acid units esterified with glycerol or ω-hydroxyfatty acids (Fig. 14.3). whereas the second is a lignin-like polymer, spatially separated from the aliphatic domains, sitting in the primary cell wall (Fig. 14.3) and composed of crosslinked hydroxycinnamic acid-based units, including amides [7, 57, 58]. The presence of polysaccharide moieties bound to the lignin-type or aliphatic domains, has also been suggested [7, 57, 58].

14.3.2 Suberin depolymerization and monomer composition

14.3.2.1 Suberin depolymerization methods

The depolymerization of suberin through ester cleavage is in general a key step both for composition analysis (usually carried out by GC–MS), and to isolate monomers for further chemical transformation. Depending either

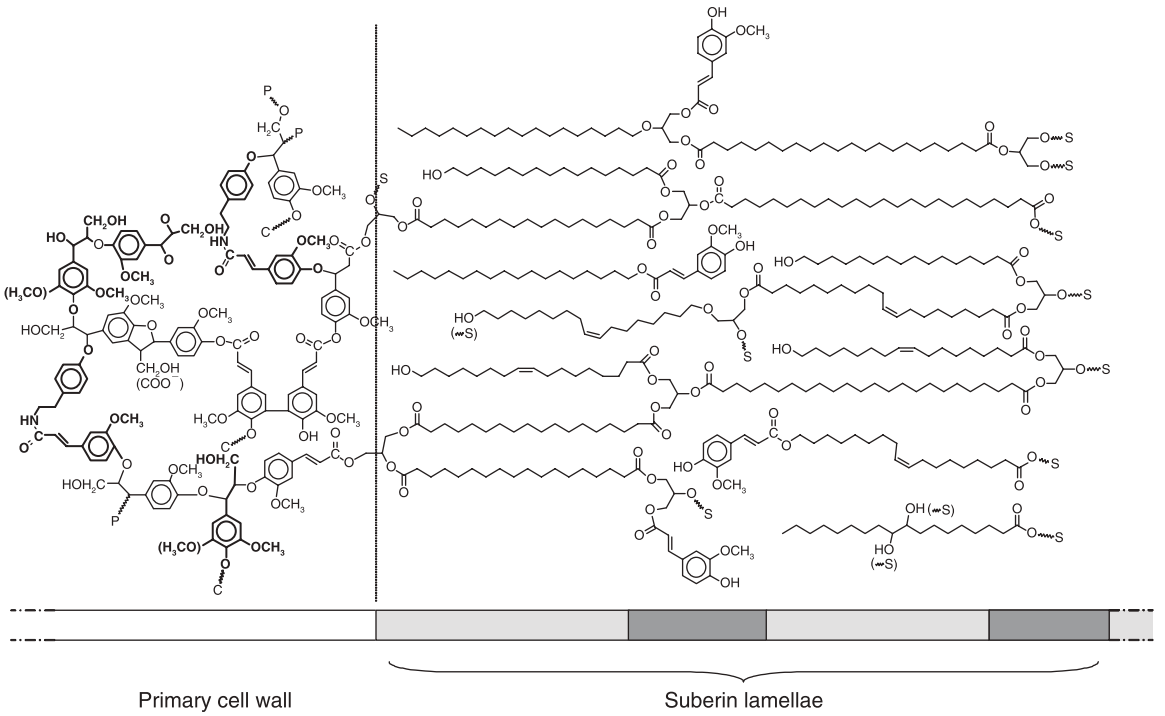

Figure 14.3 Structure proposed for suberin from potato (*Solanum tuberosum*) periderm. C: carbohydrate; P: phenolic; S: suberin. (Reprinted from Reference [1], with permission from NRC Research Press.)

on the analytical methods, or on the types of functionalities envisaged for further chemical modification, different depolymerization approaches have been implemented, involving either alkaline hydrolysis or alcoholysis (mainly methanolysis).

Alkaline methanolysis with anhydrous sodium methoxide is probably the most common depolymerization method [5, 7, 12, 14, 39, 40, 49–51, 59–66], which yields the aliphatic acid components in the form of methyl esters. Complete suberin methanolysis is normally achieved by treating it with refluxing methanol containing 3 per cent of NaOMe for about 3 h [7, 60]. However, under such conditions, epoxy moieties are, at least in part, cleaved, leading to the corresponding methoxyhydrins.

Under alkaline hydrolysis conditions, the ensuing free ω-hydroxyfatty acids and α,ω-dicarboxylic acids are obtained, with extensive cleavage of epoxy functionalities to the corresponding *vic*-diols. It has been claimed, however, that when the reaction is carried out for 15 min using KOH in ethanol:water 9:1 v:v, total depolymerization occurs with preservation of the epoxy functionalities [63], a feature that can be interesting for some polymer applications.

Methanolysis under mild conditions has been studied mainly for structural elucidation purposes [7, 51, 60]. However, such conditions can also be considered for the preferential isolation of specific groups of components, such as the alkanoic and α,ω-dicarboxylic acids generally involved in more labile structures [7,51,60]. Finally, methanolysis in the presence of calcium oxide was shown to yield oligomers [39, 40, 48–50, 59, 67], which, apart from their interest for structural elucidation, might also be considered as macromonomers for other applications. However, these mild methanolysis conditions are marred by an important drawback in terms of their use as sources of monomers/macromonomers for polymer synthesis, because they generally result in quite low yields.

14.3.2.2 Suberin monomer composition

The detailed monomer distribution of suberin from several species has been recently reviewed [14]. Table 14.1 summarizes the composition of the two most relevant suberin sources in the present context (*i.e. Quercus suber* cork and *Betula pendula* outer bark) and the structures of representative elements of each group are shown in Fig. 14.4.

Table 14.1

Relative abundance of aliphatic suberin monomers from the extractive-free *Quercus suber* cork and *Betula pendula* outer bark (adapted from [14])

	Quercus suber	*Betula pendula*
References	[7, 8, 12, 51, 60, 61, 68]	[37, 63]
Aliphatic alcohols	0.4–4.7	–
Fatty acids	**2.5–14.9**	**7.4–12.3**
C(16:0)	0–0.5	–
di(OH)-C(16:0)	0–0.9	–
C(18:1)	0–1.8	–
9,10-di(OH)-C(18:0)	0–6.6	–
9,10-epoxi-C(18:0)	0–2.2	–
C(20:0)	0–0.3	–
di-OH-C(20:0)	0–10.1	–
C(22:0)	0–2.5	–
C(24:0)		–
C(26:0)	0–2	–
ω-Hydroxyfatty acids	**36.0–61.7**	**76.7–79.7**
C(16:0)	0–1.2	
9,16-di-OH(C16:0)	0	3.2–3.7
C(18:0)	0–0.6	
C(18:1)	0–18.2	11.1–12.2
9,10-epoxy-C(18:0)	0–5.5	37.0–39.2
9,10-di(OH)-C(18:0)	0–12.7	8.4–8.6
9,10-(OH,OMe)-C(18:0)	0–7.5	
C(20:0)	0–2.2	2.8
C(20:1)	0–1.2	
C(22:0)	0–28.6	13.6–13.9
C(24:0)	0–4.6	
C(26:0)	0–4.4	
α,ω-Dicarboxylic acids	**6.1–53.3**	**10.4–12.9**
C(16:0)	0–3.1	
C(18:0)	0–0.5	0–0.9
C(18:1)	0–9.1	3.4–4.7
9,10-epoxy-(C18:0)	0–37.8	
9,10-di(OH)-C(18:0)	0–7.7	
9,10-(OH,OMe)-C(18:0)	0–20	
C(20:0)	0–4.9	
C(20:1)	0–0.3	
C(22:0)	0–7.1	6.1–8.2
C(24:0)	0–1.1	
Aromatic compounds	**0.1–7.9**	–
Ferulic acid	0.1–7.9	–

As in the case of total suberin contents in its natural precursors, the monomer composition and abundance are also highly variable, but in general the most abundant families of compounds are ω-hydroxyfatty acids, followed by α,ω-dicarboxylic acids and by smaller amounts of fatty acids, aliphatic alcohols and aromatic compounds.

ω-Hydroxyalkanoic acids are generally the most important group of components, representing between 36.0–61.7 per cent and 76.7–79.7 per cent of suberin monomers from cork and birch bark, respectively. Even C-numbered chains between C16 and C26 are frequently found, and among them, the C22 and C18 are clearly dominant. In the birch outer bark suberin, the 9,10-epoxy C18 derivative is particularly abundant, together with smaller amounts of the 9,10-dihydroxy derivative. C(18:1) and C(22:0) ω-hydroxy fatty acids are also abundant in this suberin. In cork suberin, C(18:1) and C(22:0) are in general the most abundant ω-hydroxyacids, whereas the

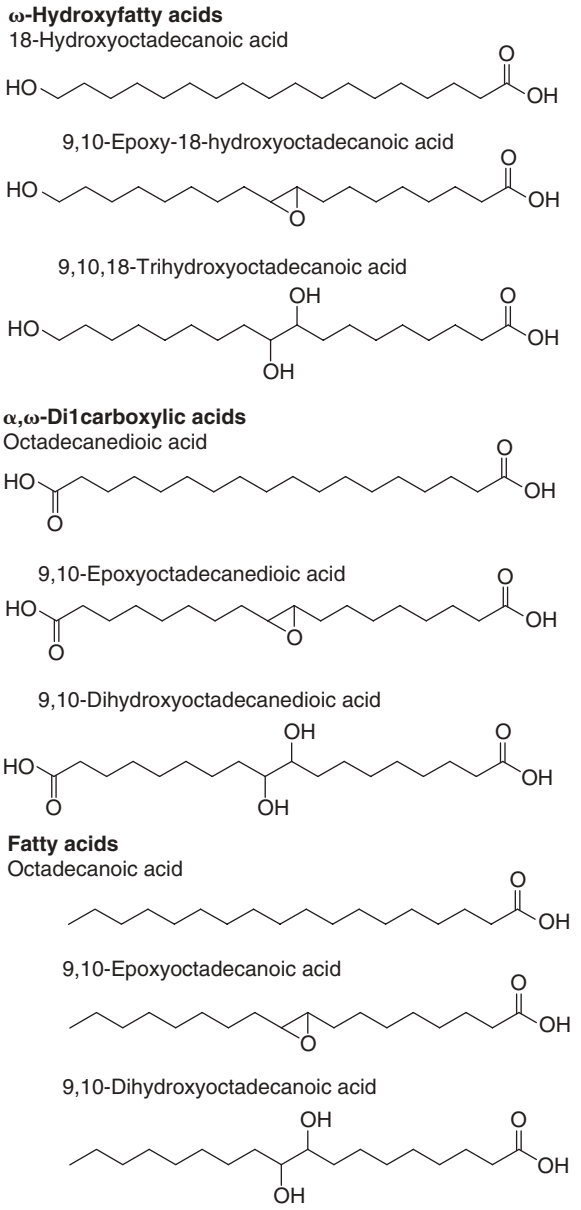

Figure 14.4 Structures of monomeric components of the most representative families of compounds formed in suberin depolymerization.

9,10-oxygenated derivatives are significantly less important than in the birch counterpart. The predominance of 9,10-dihydroxy derivative, sometimes together with the mid-chain 9,10-hydroxymethoxy derivative of the epoxide form, is certainly due to the degradation of the later under the reaction conditions used for suberin depolymerization.

α,ω-Dicarboxylic acids are generally the second most abundant group of components, representing between 6.1–53.3 per cent and 10.4–12.9 per cent of suberin monomers from cork and birch bark, respectively. Once more, the C18 and C22 structures are the prevalent components.

In the case of birch outer bark suberin, only the C(18:1) and C(22:0) homologues were found in considerable amounts, whereas in the case of cork suberin, the 9,10-epoxy and the corresponding 9,10-dihydroxy C18 α,ω-dicarboxylic acids are dominant.

Alkanoic acids represent a smaller fraction of suberin monomers, *viz.* 2.5–14.9 per cent and 7.4–12.3 per cent in cork and birch bark suberin, respectively. In cork, this fraction is mainly composed of saturated even-numbered homologues, ranging from C16 to C26, with the C18 (including the 9,10-oxygenated derivatives) and C22 as the dominant components.

Aliphatic alcohols represent less than 5 per cent of cork suberin and were not reported in the birch bark counterpart. C22 and C24 homologues are the most abundant components of this fraction.

Finally, a small fraction of aromatic compounds was also detected in cork suberin, with ferulic acid as the dominant component.

As a general overview, crucial for tailoring applications of suberin monomers in polymer synthesis, it can be concluded that C18, followed by C22 monomers, are the dominant aliphatic compounds and that most of them are carboxylic acids, bearing at least one aliphatic-OH functionality. Additionally, the 9,10-epoxide functionality is also very common.

Another important issue that should be addressed considering future applications of suberin momomers, is their absolute abundance in the hydrolysis/methanolysis mixtures. In fact, most studies have only looked at their relative abundance, and the total amount of monomers detected, relative to the mass of depolymerized suberin, is seldom provided. However, values between 27 and 74 per cent have been reported for *Quercus suber* cork [7, 8, 51], clearly showing that a non-negligible percentage of suberin is frequently *not detected* by GC–MS analysis. The exact nature of such an undetected fraction is still unknown, but it should obviously be composed by non-volatile high molecular weight components resistant to alkaline hydrolysis/methanolysis [7, 8]. It might, however, be speculated that this fraction could be composed of suberan-type oligomers. Suberan is a non-hydrolyzable highly aliphatic macromolecule found in the periderm tissue of some angiosperm species [69], whose inertness explains its detection in forest soils and fossilized samples [70, 71].

To the best of our knowledge, no such approach has been carried out for birch outer bark suberin.

In conclusion, well-established methodologies [72–74] for the selective isolation/purification of the most abundant suberin components are obviously extremely valuable for the commercial exploitation of suberin as a source of new macromolecular materials.

14.4 APPLICATIONS OF SUBERIN AND SUBERIN COMPONENTS

Although the composition of the aliphatic suberin has been thoroughly studied for many plant species [14], the physical properties of the ensuing mixtures of monomeric components, as well as their use as raw materials for polymer synthesis, are still poorly studied topics. The following sections provide a general overview of the published data on the physical properties of depolymerized suberin mixtures and on their applications in the synthesis of polymeric materials.

The only suberin depolymerization mixtures which were reported to have been used for functional characterization and for the synthesis of polymeric materials were obtained from *Quercus suber* cork, with the exception of a recent study where the reactive monomer was isolated from birch outer bark suberin [75].

14.4.1 Physical properties of depolymerized suberin

The only thorough characterization of mixtures of depolymerized suberin components (*dep-suberin*) was carried out by us in a comprehensive investigation of this material. The opaque pasty samples were obtained by the alkaline methanolysis of *Quercus suber* L. cork [8,76]. Under the conditions used for the depolymerization, most of the carboxylic acid functions were therefore converted into the corresponding methyl esters.

Given the predominance of long aliphatic chains in most of its components, which indeed impart to cork its well-known and largely exploited hydrophobic character, it seemed interesting to assess the surface properties of *dep-suberin*. A detailed study was therefore carried out using various techniques [76]. The surface energy of the solid (pasty) *dep-suberin* at 25°C, determined from contact angle measurements with liquids of different polarity, applying the Owens–Wendt approach, was $42\,mJ\,m^{-2}$, with a polar component of about $4\,mJ\,m^{-2}$. Measurements of the surface tension of the liquid samples at 50–110°C, gave a linear variation of γ with temperature, with an extrapolated value of $37\,mJ\,m^{-2}$ at 25°C. This difference was attributed to the microcrystalline character of the solid sample (see below), associated with a higher cohesive energy and, hence, a higher surface energy. Since a

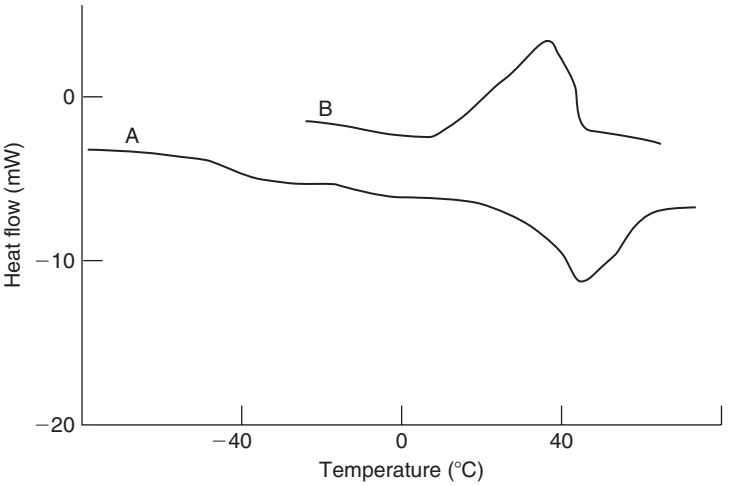

Figure 14.5 DSC thermograms of suberin. A: heating; B: cooling. (Reprinted from Reference [78], with permission from Elsevier.)

mixture of alkanes with the same range of chain lengths as the *dep-suberin* components would display a surface energy close to 28 mJ m^{-2}, it follows that (i) some of the polar groups in those components were present on the *dep-suberin* surface, as confirmed by the modest, but non-negligible, polar contribution to the surface energy, and (ii) some intermolecular interactions, mostly through hydrogen bonding, induced an increase in cohesive energy, compared with the purely dispersive alkane structures, as suggested by the correspondingly higher γ_d values obtained by both contact angle and inverse chromatography measurements [76]. Notwithstanding these fine-tuned considerations, *dep-suberin* must be considered as a rather non-polar material with surface properties resembling those of its cork precursor, whose reported values of surface energy range between 30 and 40 mJ m^{-2}[77].

The differential scanning calorimetry (DSC) tracings of *dep-suberin* (see Fig. 14.5) showed that annealing a molten sample in liquid nitrogen, produced an amorphous material with a glass transition temperature of about −50°C, which crystallized when brought to about 30°C [78]. The melting temperature of the microcrystalline phase was centred at about 40°C (broad endothermic peak). These observations were confirmed by optical microscopy carried out with reproducible temperature cycles between −20°C and 80°C [79]. A quantitative assessment of the birefringence (Fig. 14.6) showed a constant maximum value (heating cycle) up to about 0ºC, followed by a gradual decrease to zero birefringence at about 50°C. The cooling cycle reproduced the same features in reverse. The images captured in this context showed dense microcrystalline domains within an amorphous matrix [79].

Given the broad temperature range associated with the melting or forming of these crystalline phases, and the very small size of the crystals, it seems likely that the *dep-suberin* components more apt to crystallize because of their suitable structures, do so on an individual basis at their respective freezing temperature, when the liquid mixture is slowly cooled down. The result is therefore a set of microcrystals, each member belonging to a given *dep-suberin* component. Interestingly, the fact of having a rather complex mixture of compounds does not hinder the individual crystallization of some of them, most probably because the major driving force is associated with the ease of self-assembly among their *long and linear* aliphatic sequences.

The characteristic whitish and pasty appearance of these *dep-suberin* samples at room temperature reflects therefore the combination of a viscous liquid containing a substantial proportion of microcrystals.

The densities of these *dep-suberin* samples were surprisingly high, *viz. ca.* 1.08 at room temperature and above unity even up to 55°C [78], compared with those of alkanes of similar chain length, which are about 0.8 at room temperature. This clearly confirmed the existence of additional intermolecular interactions through hydrogen bonding from the OH groups borne by the different monomeric structures. Indeed, fatty acid esters, as well as fatty alcohols and diols, have densities close to those measured for *dep-suberin* in that work [78].

The thermogravimetry (TGA) of *dep-suberin* in a nitrogen atmosphere [78] showed a good thermal stability up to about 280°C, followed by a progressive weight loss, reaching a plateau at about 80 per cent volatilization at 470°C and leaving a carbonaceous residue.

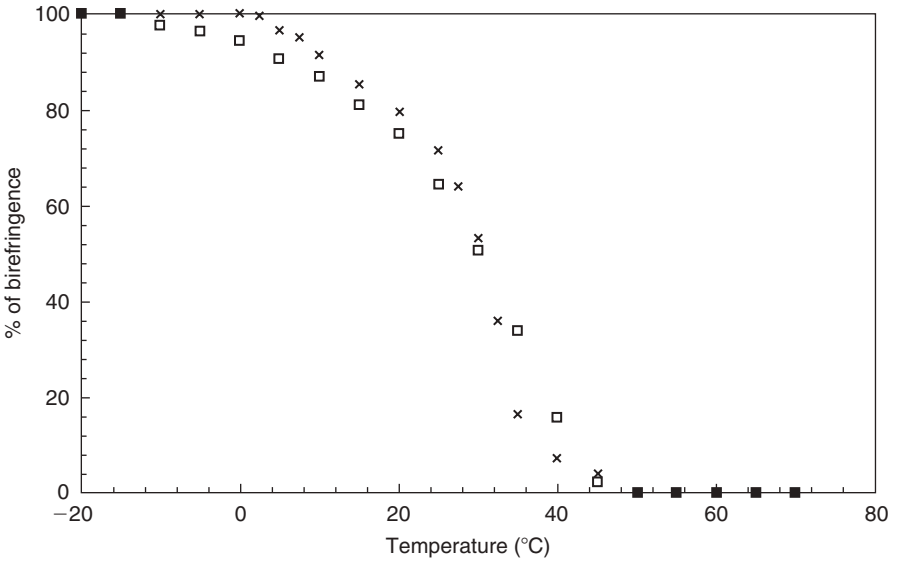

Figure 14.6 Melting (x) and recrystallization [□] of suberin, as observed by the change in birefringence intensity, as a function of temperature (the 100 per cent birefringence refers to the *maximum extent* of crystallization and *not* to the actual percentage of the crystalline phase). (Reprinted from Reference [79], with permission from Elsevier.)

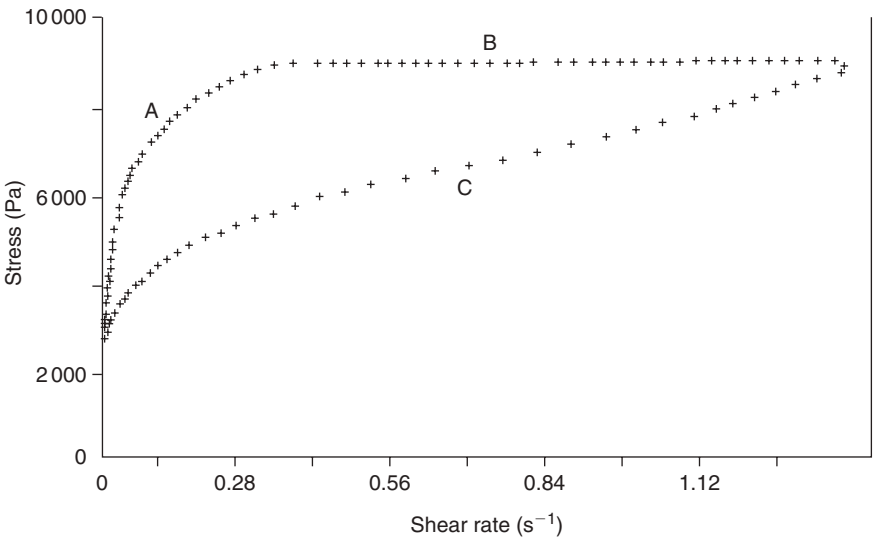

Figure 14.7 A Typical rheogram of suberin at 20ºC. A: increasing stress; B: constant stress; C: decreasing stress. (Reprinted from Reference [78], with permission from Elsevier.)

The rheological properties of *dep-suberin* at room temperature were typical of a plastic response, with an important yield-stress value and a thixotropic behaviour, as shown in Fig. 14.7 [78, 80]. These features are usually associated with either intermolecular or interphase shear-induced destructuration (or both), followed by a time-dependent restructuration at rest. Since *dep-suberin* was characterized by both intermolecular association through hydrogen bonding and the existence of a liquid/crystal interphase at room temperature, its rheological study was extended to higher temperatures. The extent of yield stress decreased drastically as the temperature was raised and indeed vanished at about 50°C (*i.e*. when all the microcrystals had melted). Moreover, the rheogram at this temperature became linear, *viz*. liquid *dep-suberin* displayed a Newtonian behaviour. These observations, shown in

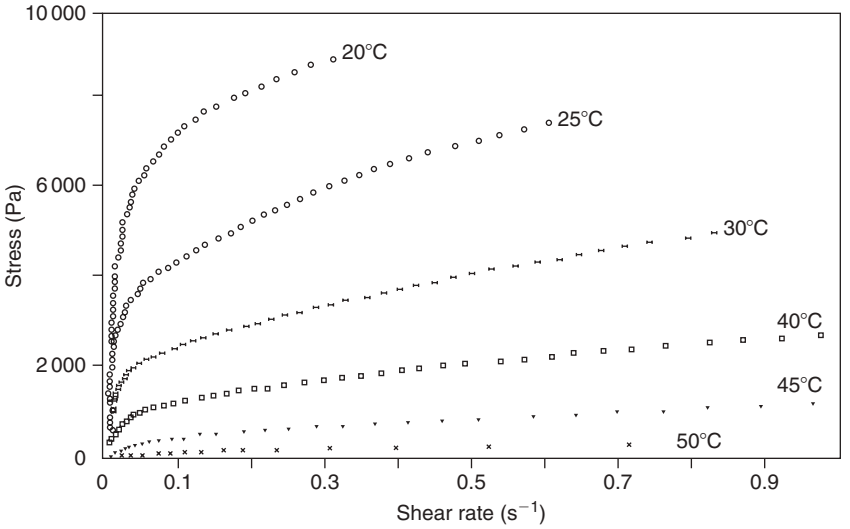

Figure 14.8 Rheograms of suberin at different temperatures (increasing stress mode). (Reprinted from Reference [78], with permission from Elsevier.)

Fig. 14.8, revealed that the major cause of its plastic behaviour at room temperature was its heterogeneous nature and the consequent strong interfacial interactions between the liquid and the microcrystals [78].

The actual values of the viscosity varied dramatically with temperature, going from 14 000 to 0.18 Pa s between 20°C and 65°C [80]. The corresponding Eyring plot [80] showed three distinct regimes (Fig. 14.9): (i) below 37°C, the presence of the microcrystalline phase induced a high value of the flow activation energy ($E_a = 88\,kJ\,mol^{-1}$); (ii) above 55°C, where the sample was a homogeneous liquid, E_a dropped to $34\,kJ\,mol^{-1}$; (iii) a transition zone between these two temperatures, reflected the progressive melting of the microcrystals, which gave rise to a continuous change in the substrate solid–liquid contents and physical consistency.

Tack measurements [80] showed that the dynamic resistance of *dep-suberin* to film splitting decreased, as expected, with both increasing temperature and increasing shear rate. The temperature effect reflected mostly the melting of the crystalline phase, since the drop in tack was quite drastic between 30°C and 50°C (the melting range). The tack value registered in each test was constant with respect to time during up to 20 min.

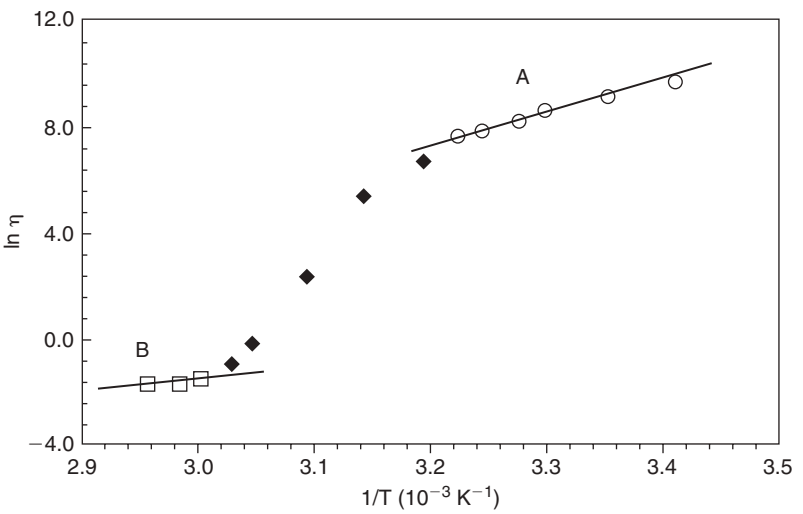

Figure 14.9 Eyring plot related to the viscous flow of suberin. (Reprinted from Reference [80], with permission from Elsevier.)

14.4.2 Depolymerized suberin mixtures as a functional additive

The microcrystalline character of *dep-suberin* mixtures described above, prompted us to examine the possible role of this material as an additive to offset printing inks, in replacement of other waxy materials like polytetrafluoroethylene (PTFE) oligomers [80]. Two reference inks were employed for this study, namely a typical vegetable-oil-based commercial ink and a 'waterless' ink containing petroleum-based diluents, to both of which *dep-suberin* was added in proportions of 2 to 10 per cent w/w. The characterization of these formulations included the determination of tack and viscosity, as well as printing tests. The presence of *dep-suberin* in the waterless ink only affected its bulk properties, by stabilizing the tack value with time and inducing a modest decrease in viscosity (with 10 per cent *dep-suberin*), without any detectable modification of the surface properties. This suggests that the hydrocarbon diluent of that ink acted as a good solvent for the *dep-suberin*, which therefore did not migrate to the surface of the printed film. With the more conventional vegetable-oil ink, *dep-suberin* induced a significant decrease in tack, small changes in viscosity and a two-fold decrease in the gloss of a printed film containing 10 per cent of it. The latter result clearly showed that at least part of the crystalline components of *dep-suberin* were not dissolved in the ink medium and could therefore migrate to the surface to produce the desired change in its optical properties.

14.4.3 Polymers from suberin monomers

Little has been published on the use of the suberin depolymerization products as monomers for the synthesis of novel macromolecular materials, which has so far concentrated on polyurethanes and polyesters using the mixture of aliphatic monomers.

14.4.3.1 Synthesis of polyurethanes

In a preliminary study also involving model compounds [81], the kinetics of urethane formation was followed by FTIR spectroscopy using an aliphatic and an aromatic monoisocyanate and their homologous diisocyanates. Both the model reactions and the polymer synthesis gave clear cut second-order behaviour, indicating that the hydroxyl groups borne by the suberin monomers displayed conventional aliphatic-OH reactivity.

The subsequent investigation [79] concentrated on the polymerization conditions and the thorough characterization of the ensuing polyurethanes, prepared using both aliphatic and aromatic diisocyanates. When the initial [NCO]/[OH] molar ratio was unity, all the polymers gave about 30 per cent of soluble material, the rest being a crosslinked product. This systematic result suggested that, on the one hand, some of the suberin monomers had a functionality higher than two, thus promoting a non-linear polycondensation leading to about 70 per cent of gel, and, on the other hand, monofunctional components must have been present in the monomer mixture, which played the role of chain-growth terminators, giving rise to the sol fraction. This conclusion was corroborated by the fact that the FTIR spectra of both fractions were practically identical, as shown in Fig. 14.10, suggesting that the solubility/insolubility factor was not based on differences in the polymer chemical structure, but instead on its macromolecular architecture.

The T_g of these polyurethanes [81] followed classical trends in that, for the networks, the use of aromatic diisocyanates resulted in high values (about 100ºC) associated with the stiffness of their moieties, whereas with the aliphatic counterparts, values around room temperature indicated much higher chain flexibility. The T_gs of the soluble fractions were much lower than those of their corresponding crosslinked materials, which is in tune with the presence of very mobile open-ended branches, generated by the insertion of monofunctional monomers into the polymer structure.

14.4.3.2 Synthesis of polyesters

Benitez *et al*. [82], recently reported the synthesis of a polyester resembling cutin, a natural polymer whose structure is close to that of aliphatic suberin [83], by a circular approach, which consisted in depolymerizing cutin through ester cleavage and then submitting the ensuing monomer mixture to a chemical polyesterification process.

The crosslinked material they obtained displayed, as one would indeed expect, very similar spectroscopic features compared with those of the starting cutin. In a subsequent study in the same vein [84], glycerol derivatives of mono- and dicarboxylic acids, whose structure simulated those present in both suberin and cutin, were prepared and characterized in an effort to simulate the biological synthesis of those natural polymers and exploit their peculiar properties, particularly their tendency to form supramolecular assemblies.

In another recent study [75], 9,10-epoxy-18-hydroxyoctadecanoic acid, isolated from birch outer bark suberin was used as starting material for the lipase-catalyzed synthesis of an epoxy-functionalized polyester. Immobilized

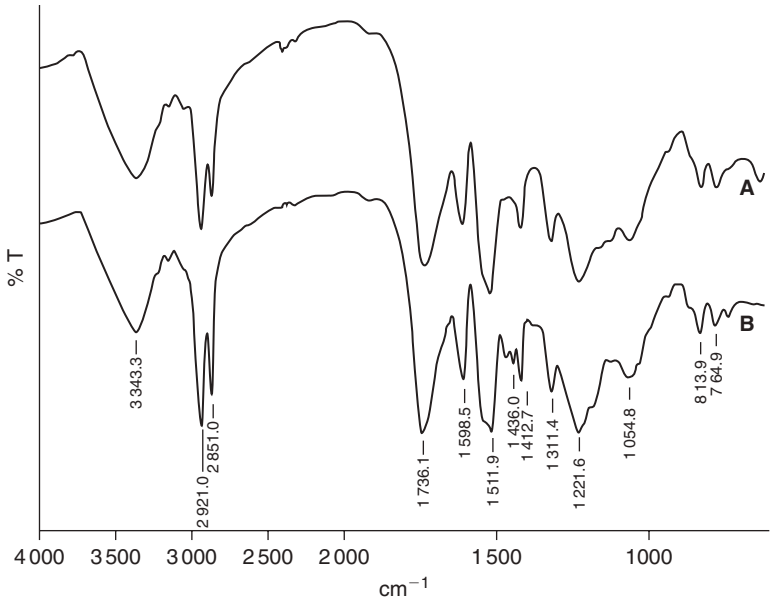

Figure 14.10 FTIR spectra of a polyurethane prepared from suberin and MDI-2.0 with $[NCO]_0/[OH]_0 = 1$. A: insoluble fraction; B: soluble fraction. (Reprinted from Reference [79], with permission from Elsevier.)

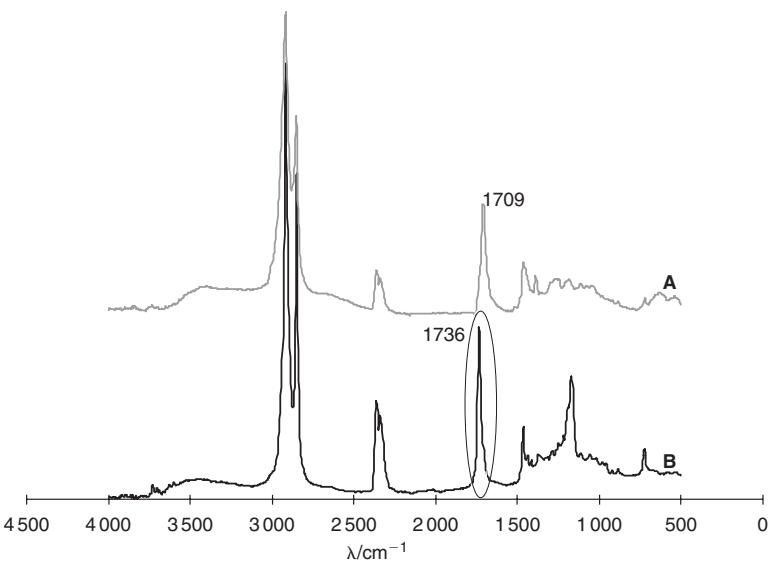

Figure 14.11 FTIR spectra of A: *dep-suberin* and B: the polyester formed by its polycondensation.

Candida antarctica lipase B was shown to catalyse efficiently the polycondensation of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid, yielding epoxy-functionalized polyesters with Mw values ranging from 15 000 to 20 000.

Another promising approach, in the sense that it avoids expensive fractionation procedures, is to promote the direct polymerization of *dep-suberin* mixtures under polycondensation or polytransesterification conditions, using *dep-suberin* mixtures issued from alkaline hydrolysis or methanolysis, respectively. Ongoing experiments prove that both types of systems are viable [85, 86], as shown by the FTIR spectrum of the product obtained from a polycondensation of a *dep-suberin* (Fig. 14.11), which clearly shows the feature of a polyester. This evidence was further corroborated by NMR spectroscopy and molecular weight measurements [85].

14.5 CONCLUDING REMARKS

The main purpose of this chapter was to emphasize the interest in considering cork and suberin, both cheap renewable resources, potentially available in very large amounts, as valuable precursors to novel macromolecular materials, particularly when exploited as by-products. Undoubtedly, the predominance of long aliphatic moieties in both these precursors, points to applications of the corresponding polymers associated with a relative softness and a highly hydrophobic character, but these obvious features should not be considered as exclusive since much more needs to be explored in this still young domain.

REFERENCES

1. Bernards M.A., Demystifying suberin, *Can. J. Bot.*, **80**(3), 2002, 227–240.
2. Silva S.P., Sabino M.A., Fernandes E.M., Correlo V.M., Boesel L.F., Reis R.L., Cork: Properties, capabilities and applications, *Int. Mater. Rev.*, **50**(6), 2005, 345–365.
3. Ciesla W.M., *Non-Wood Forest Products from Temperate Broad-Leaved Trees*, FAO, Rome, 2002.
4. CorkMasters, www.corkmasters.com (browsed May, 2007).
5. Pereira H., Chemical-Composition and Variability of Cork from *Quercus-suber* L., *Wood Sci. Technol.*, **22**(3), 1988, 211–218.
6. Lopes M., Neto C.P., Evtuguin D., Silvestre A.J.D., Gil A., Cordeiro N., Gandini A., Products of the permanganate oxidation of cork, desuberized cork, suberin and lignin from *Quercus suber* L., *Holzforschung*, **52**(2), 1998, 146–148.
7. Lopes M.H., Gil A.M., Silvestre A.J.D., Neto C.P., Composition of suberin extracted upon gradual alkaline methanolysis of *Quercus suber* L. cork, *J. Agric. Food Chem.*, **48**(2), 2000, 383–391.
8. Cordeiro N., Belgacem M.N., Silvestre A.J.D., Neto C.P., Gandini A., Cork suberin as a new source of chemicals. 1. Isolation and chemical characterization of its composition, *Int. J. Biol. Macromol.*, **22**(2), 1998, 71–80.
9. Conde E., Cadahia E., Garcia-Vallejo M.C., de Simon B.F., Polyphenolic composition of *Quercus suber* cork from different Spanish provenances, *J. Agric. Food Chem.*, **46**(8), 1998, 3166–3171.
10. Conde E., Cadahia F., GarciaVallejo M.C., deSimon B.F., Adrados J.R.G., Low molecular weight polyphenols in cork of *Quercus suber*, *J. Agric. Food Chem.*, **45**(7), 1997, 2695–2700.
11. Conde E., Cadahia E., Garcia-Vallejo M.C., Gonzalez-Adrados J.R., Chemical characterization of reproduction cork from Spanish *Quercus suber*, *J. Wood Chem. Technol.*, **18**(4), 1998, 447–469.
12. Conde E., Garcia-Vallejo M.C., Cadahia E., Variability of suberin composition of reproduction cork from *Quercus suber* throughout industrial processing, *Holzforschung*, **53**(1), 1999, 56–62.
13. Cadahia E., Conde E., de Simon B.F., Garcia-Vallejo M.C., Changes in tannic composition of reproduction cork *Quercus suber* throughout industrial processing, *J. Agric. Food Chem.*, **46**(6), 1998, 2332–2336.
14. Gandini A., Pascoal C., Silvestre A.J.D., Suberin: A promising renewable resource for novel macromolecular materials, *Progr. Polym. Sci.*, **31**(10), 2006, 878–892.
15. Rosa M.E., Osorio J., Green V., Fortes M.A., Torsion of cork under compression. in *Advanced Materials Forum Ii*, Ed. 2004, pp. 235–238.
16. Parralejo A.D., Guiberteau F., Fortes M.A., Rosa M.F., Mechanical properties of cork under contact stresses, *Rev. de Metalurgia*, **37**(2), 2001, 330–335.
17. Vaz M.F., Fortes M.A., Friction properties of cork, *J. Mater. Sci.*, **33**(8), 1998, 2087–2093.
18. Pina P., Fortes M.A., Characterization of cells in cork, *J. Phys. D: Appl. Phys.*, **29**(9), 1996, 2507–2514.
19. Rosa M.E., Fortes M.A., Water-absorption by cork, *Wood Fiber Sci.*, **25**(4), 1993, 339–348.
20. Rosa M.E., Fortes M.A., Deformation and fracture of cork in tension, *J. Mater. Sci.*, **26**(2), 1991, 341–348.
21. Rosa M.E., Fortes M.A., Temperature-induced alterations of the structure and mechanical-properties of cork, *Mater. Sci. Eng.*, **100**, 1988, 69–78.
22. Rosa M.E., Fortes M.A., Stress-relaxation and creep of cork, *J. Mater. Sci.*, **23**(1), 1988, 35–42.
23. Gibson L.J., Easterling K.E., Ashby M.F., The structure and mechanics of cork, *Proc. Royal Soc. London Series A-Math. Phys. Eng. Sci.*, **377**(1769), 1981, 99–117.
24. Gameiro C.P., Cirne J., Gary G., Experimental study of the quasi-static and dynamic behaviour of cork under compressive loading, *J. Mater. Sci.*, **42**, 2007, 4316–4324.
25. Amorim Industrial solutions, http://www.amorimsolutions.com/ (browsed May, 2007).
26. Shuttle's Cork From Portugal, *New York Times*, November, 3, 1981.
27. Thermal protection systems for space vehicles, www.p2pays.org/ref/34/33161.pdf (browsed May, 2007).
28. Airbus fait appel au groupe portugais Amorim pour fournir du liège aggloméré. http://www.bulletins-electroniques.com/actualites/43153.htm. (browsed June, 2007).

29. Karade S.R., Irle M., Maher K., Influence of granule properties and concentration on cork-cement compatibility, *Holz Als Roh-Und Werkstoff*, **64**(4), 2006, 281–286.
30. Gil L., *Cortiça, Produção Tecnologia e Aplicação*, INETI, Lisbon, 1988.
31. Gil L., Cork powder waste: An overview, *Biomass & Bioenergy*, **13**(1–2), 1997, 59–61.
32. Kamm B., Gruber P.R., Kamm M., *Biorefineries – Industrial Processes and Products*, Wiley-VCH, Weinheim, 2006.
33. Sousa A.F., Pinto P., Silvestre A.J.D., Neto C.P., Triterpenic and other lipophilic components from industrial cork byproducts, *J. Agric. Food Chem.*, **54**(18), 2006, 6888–6893.
34. Evtiouguina M., Barros-Timmons A., Cruz-Pinto J.J., Neto C.P., Belgacem M.N., Gandini A., Oxypropylation of cork and the use of the ensuing polyols in polyurethane formulations, *Biomacromolecules*, **3**(1), 2002, 57–62.
35. Evtiouguina M., Barros A.M., Cruz-Pinto J.J., Neto C.P., Belgacem N., Pavier C., Gandini A., The oxypropylation of cork residues: Preliminary results, *Bioresource Technol.*, **73**(2), 2000, 187–189.
36. Evtiouguina M., Gandini A., Neto C.P., Belgacem N.M., Urethanes and polyurethanes based on oxypropylated cork: 1. Appraisal and reactivity of products, *Polym. Int.*, **50**(10), 2001, 1150–1155.
37. Ekman R., The suberin monomers and triterpenoids from the outer bark of *betula-verrucosa* ehrh, *Holzforschung*, **37**(4), 1983, 205–211.
38. Holloway P.J., Some variations in the composition of suberin from the cork layers of higher-plants, *Phytochemistry*, **22**(2), 1983, 495–502.
39. Graca J., Pereira H., Glyceryl–acyl and aryl–acyl dimers in Pseudotsuga menziesii bark suberin, *Holzforschung*, **53**(4), 1999, 397–402.
40. Graca J., Pereira H., Suberin structure in potato periderm: Glycerol, long-chain monomers, and glyceryl and feruloyl dimers, *J. Agric. Food Chem.*, **48**(11), 2000, 5476–5483.
41. Christie W.W., The lipid library. http://www.lipidlibrary.co.uk/ (browsed January, 2006).
42. Bernards M.A., Lewis N.G., The macromolecular aromatic domain in suberized tissue: A changing paradigm, *Phytochemistry*, **47**(6), 1998, 915.
43. Kolattukudy P.E., Bio-polyester membranes of plants – cutin and suberin, *Science*, **208**(4447), 1980, 990–1000.
44. Kolattukudy P.E., Polyesters in higher plants, in *Advances in Biochemical Engineering/Biotechnology: Biopolyesters,* Eds.: Babel W. and Steinbüchel A., Springer, Berlin, Heidelberg, 2001.
45. Kolattukudy P.E., Espelie K.E., Chemistry, biochemistry, and function of suberin and associated waxes, in *Natural products of woody plants, chemical extraneous to the lignocellulosic cell wall,* Ed.: Rowe J., Springer, Berlin, Heidelberg, 1989.
46. Gil A.M., Lopes M., Rocha J., Neto C.P., A C-13 solid state nuclear magnetic resonance spectroscopic study of cork cell wall structure: The effect of suberin removal, *Int. J. Biol. Macromol.*, **20**(4), 1997, 293–305.
47. Lopes M.H., Sarychev A., Neto C.P., Gil A.M., Spectral editing of C-13 CP/MAS NMR spectra of complex systems: Application to the structural characterisation of cork cell walls, *Solid State Nuc. Magn. Reson.*, **16**(3), 2000, 109–121.
48. Graca J., Santos S., Suberin: A biopolyester of plants' skin, *Macromol. Biosc.*, **7**(2), 2007, 128–135.
49. Graca J., Pereira H., Cork suberin: A glyceryl based polyester, *Holzforschung*, **51**(3), 1997, 225–234.
50. Graca J., Pereira H., Diglycerol alkenedioates in suberin: Building units of a poly(acylglycerol) polyester, *Biomacromolecules*, **1**(4), 2000, 519–522.
51. Graca J., Pereira H., Methanolysis of bark suberins: Analysis of glycerol and acid monomers, *Phytochem. Anal.*, **11**(1), 2000, 45–51.
52. Graca J., Schreiber L., Rodrigues J., Pereira H., Glycerol and glyceryl esters of omega-hydroxyacids in cutins, *Phytochemistry*, **61**(2), 2002, 205–215.
53. Gil A.M., Lopes M.H., Neto C.P., Callaghan P.T., An NMR microscopy study of water absorption in cork, *J. Mater. Sci.*, **35**(8), 2000, 1891–1900.
54. Garbow J.R., Ferrantello L.M., Stark R.E., 13C Nuclear magnetic resonance study of suberized potato cell wall, *Plant Physiol.*, **90**, 1989, 783–787.
55. Stark R.E., Garbow J.R., Nuclear magnetic resonance relaxation studies of plant polyester dynamics. 2. Suberized potato cell walls, *Macromolecules*, **25**, 1992, 149–154.
56. Yan B., Stark R.E., Biosynthesis, molecular structure, and domain architecture of potato suberin: A C-13 NMR study using isotopically labeled precursors, *J. Agric. Food Chem.*, **48**(8), 2000, 3298–3304.
57. Neto C.P., Cordeiro N., Seca A., Domingues F., Gandini A., Robert D., Isolation and characterization of a lignin-like polymer of the cork of *Quercus suber* L., *Holzforschung*, **50**(6), 1996, 563–568.
58. Sitte P., Zum Feinbau Der Suberinschichten Im Flaschenkork, *Protoplasma*, **54**(4), 1962, 555–559.
59. Graca J., Pereira H., Feruloyl esters of omega-hydroxyacids in cork suberin, *J. Wood Chem. Technol.*, **18**(2), 1998, 207–217.
60. Bento M.F., Pereira H., Cunha M.A., Moutinho A.M.C., van den Berg K.J., Boon J.J., van den Brink O., Heeren R.M.A., Fragmentation of suberin and composition of aliphatic monomers released by methanolysis of cork from *Quercus suber* L., analysed by GC–MS, SEC and MALDI–MS, *Holzforschung*, **55**(5), 2001, 487–493.

61. GarciaVallejo M.C., Conde E., Cadahia E., deSimon B.F., Suberin composition of reproduction cork from *Quercus suber*, *Holzforschung*, **51**(3), 1997, 219–224.
62. Arno M., Serra M.C., Seoane E., Methanolysis of cork suberin – Identification and estimation of their acidic components as methyl-esters, *Anales De Quimica Serie C-Quimica Organica Y Bioquimica*, **77**(1), 1981, 82–86.
63. Ekman R., Eckerman C., Aliphatic carboxylic-acids from suberin in birch outer bark by hydrolysis, methanolysis, and alkali fusion, *Paperi Ja Puu-Paper and Timber*, **67**(4), 1985, 255.
64. Holloway P.J., Deas A.H.B., Epoxyoctadecanoic acids in plant cutins and suberins, *Phytochemistry*, **12**(7), 1973, 1721–1735.
65. Holloway P.J., Martin J.T., Baker E.A., Chemistry of plant cutins and suberins, *Anales De Quimica-International Edition*, **68**(5–6), 1972, 905.
66. Seoane E., Serra M.C., Agullo C., 2 New epoxy-acids from cork of *Quercus-suber*, *Chem. Ind.*, **15**, 1977, 662–663.
67. Graca J., Santos S., Linear aliphatic dimeric esters from cork suberin, *Biomacromolecules*, **7**(6), 2006, 2003–2010.
68. Bento M.F., Pereira H., Cunha M.A., Moutinho A.M.C., van den Berg K.J., Boon J.J., Thermally assisted transmethylation gas chromatography mass spectrometry of suberin components in cork from *Quercus suber* L., *Phytochem. Anal.*, **9**(2), 1998, 75–87.
69. Tegelaar E.W., Hollman G., Vandervegt P., Deleeuw J.W., Holloway P.J., Chemical characterization of the periderm tissue of some angiosperm species – Recognition of an insoluble, nonhydrolyzable, aliphatic biomacromolecule (uberan), *Org. Geochem.*, **23**(3), 1995, 239–251.
70. Nierop K.G.J., Origin of aliphatic compounds in a forest soil, *Org. Geochem.*, **29**(4), 1998, 1009–1016.
71. Augris N., Balesdent J., Mariotti A., Derenne S., Largeau C., Structure and origin of insoluble and non-hydrolyzable, aliphatic organic matter in a forest soil, *Org. Geochem.*, **28**(1–2), 1998, 119–124.
72. Ekman R., Eckerman C., Mattila T., Suokas E. in *Method for converting vegetable material into chemicals*, Ed. 4732708 U.S.P., 1988.
73. Krasutsky P.A., Carlson R.M., Kolomitsyn I.V., in *Isolation of natural products from birch bark*, Ed. 20030109727 U.S.P., 2003.
74. Krasutsky P.A., Carlson R.M., Nesterenko V.V., Kolomitsyn I.V., Edwardson C.F., in *Birch bark processing and the isolation of natural products from birch bark* Ed. 6815553 U.S.P., 2004.
75. Olsson A., Lindstrom M., Iversen T., Lipase-catalyzed synthesis of an epoxy-functionalized polyester from the suberin monomer *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid, *Biomacromolecules*, **8**(2), 2007, 757–760.
76. Cordeiro N., Aurenty P., Belgacem M.N., Gandini A., Neto C.P., Surface properties of suberin, *J. Colloid Interface Sci.*, **187**(2), 1997, 498–508.
77. Cordeiro N., Neto C.P., Gandini A., Belgacem M.N., Characterization of the cork surface by inverse gas-chromatography, *J. Coll. Interface Sci.*, **174**(1), 1995, 246–249.
78. Cordeiro N., Belgacem N.M., Gandini A., Neto C.P., Cork suberin as a new source of chemicals: 2. Crystallinity, thermal and rheological properties, *Bioresource Technol.*, **63**(2), 1998, 153–158.
79. Cordeiro N., Belgacem M.N., Gandini A., Neto C.P., Urethanes and polyurethanes from suberin 2: Synthesis and characterization, *Ind. Crops Prod.*, **10**(1), 1999, 1–10.
80. Cordeiro N., Blayo A., Belgacem N.M., Gandini A., Neto C.P., LeNest J.F., Cork suberin as an additive in offset lithographic printing inks, *Ind. Crops Prod.*, **11**(1), 2000, 63–71.
81. Cordeiro N., Belgacem M.N., Gandini A., Neto C.P., Urethanes and polyurethanes from suberin. 1. Kinetic study, *Ind. Crops Prod.*, **6**(2), 1997, 163–167.
82. Beniitez J.J., Garcia-Segura R., Heredia A., Plant biopolyester cutin: A tough way to its chemical synthesis, *Biochim. Biophys. Acta-Gen. Subj.*, **1674**(1), 2004, 1–3.
83. Schreiber L., Franke R., Hartmann K.D., Ranathunge K., Steudle E., The chemical composition of suberin in apoplastic barriers affects radial hydraulic conductivity differently in the roots of rice (Oryza sativa L. cv. IR64) and corn (Zea mays L. cv. Helix), *J. Exp.. Bot.*, **56**(415), 2005, 1427–1436.
84. Douliez J.P., Barrault J., Jerome F., Heredia A., Navailles L., Nallet F., Glycerol derivatives of cutin and suberin monomers: Synthesis and self-assembly, *Biomacromolecules*, **6**(1), 2005, 30–34.
85. Sousa A.F., Pinto P.C.R.O., Silvestre A.J.D., Gandini A., Pascoal Neto C., *BIOPOL – 2007: 1st International conference on biodegradable polymers and sustainable composites*, Alicante, Spain, October 2007.

– 15 –

Starch: Major Sources, Properties and Applications as Thermoplastic Materials

Antonio J.F. Carvalho

ABSTRACT

This chapter reviews the general context of starch as a material. After a survey of the major sources of starch and their characteristic compositions in terms of amylase and amylopectin, the morphology of the granules and the techniques applied to disrupt them are critically examined. The use of starch for the production of polymeric materials covers the bulk of the chapter, including the major aspect of starch plasticization, the preparation and assessment of blends, the processing of thermoplastic starch (TPS), the problems associated with its degradation and the preparation of TPS composites and nanocomposites. The present and perspective applications of these biodegradable materials and the problems associated with their moisture sensitivity conclude this manuscript.

Keywords

Starch, Main sources, Macromolecular composition, Polymer structure and crystallinity, Granule disruption, Thermoplastic starch, Plasticization, Moisture uptake, Degradation, Blends, Composites

15.1 INTRODUCTION

Starch is the major carbohydrate reserve in higher plants. In contrast with cellulose that is present in dietary fibres, starch is digested by humans and represents one of the main sources of energy to sustain life. Bread, potato, rice and pasta are examples of the importance of starch in our society. Starch has also been extremely important for centuries in numerous non-food applications, for example, as glue for paper and wood [1] and as gum for the textile industry [2, 3]. Together with wood, natural fibres and leather, starch has been one of the choice materials since the inception of human technology.

Polysaccharides represent by far the most abundant biopolymers on earth, with cellulose, chitin and starch dominating. The first two are reviewed as sources of novel materials in Chapters 16–19 and 25, respectively, of this book. Starch is certainly one of the most versatile materials for potential use in polymer technology. It can be converted, on the one hand, into chemicals like ethanol, acetone and organic acids, used in the production of synthetic polymers and, on the other hand, it can produce biopolymer through fermentative processes or be hydrolyzed and employed as a monomer or oligomer. Finally, it can be grafted with a variety of reagents to produce new polymeric materials, used as such or as fillers for other polymers.

The conversion into small molecules is chemically easier for starch than for cellulose, making it an economic option to produce hydroxyl-containing compounds which can be exploited as monomers or as raw material in the production of other biopolymers like polylactic acid (PLA). This approach is in competition with other renewable resources *viz*. saccharose from sugar cane, used for the production of ethanol and biopolymers such as

poly-β-hydroxybutyrate (PHB) [4, 5], and lactic acid [6] as a source of its polymer [7]. These two polymers are reviewed herein in Chapters 21 and 22, respectively. Despite the importance of starch as a raw material for the production of chemicals and other polymers, its direct use as a renewable resource commodity is undoubtedly more economical and has been a major area of research in material science over the last few decades.

The chemical modification of starch can provide tailor-made materials and has been reviewed recently [8].

The literature concerning starch is extremely vast and its chemistry and technology have been comprehensively reviewed recently [2, 3, 8, 9]. More specifically, a renewed interest has arisen in the last decade, to convert starch into a plastic material capable of replacing petroleum-based counterparts. The main aim of this chapter is to review the applications of starch in the development of new polymeric materials in which its main macromolecular structure is preserved. The preparation of monomers and oligomers will also be briefly discussed.

15.2 MAIN SOURCES OF STARCH

Several plants are commercially used for the production of starch. The choice plant depends mainly on geographic and climatic factors and on the desired functional properties of the corresponding starch [10]. It is always possible to find a highly productive plant to produce starch whatever the climate and agricultural conditions: maize in tempered and subtropical zones, cassava (manioc or tapioca) and banana in tropical environments, rice in inundated areas and potatoes in cold climates. The main plant sources are listed in Table 15.1, together with their production for 2005 [11].

Apart from the traditional crops, cassava shows a great potential because it adapts to tropical zones and constitutes therefore an important crop in developing areas of the world. New sources of starch are also arising, such as banana [12], which yields a starch of excellent quality.

The development of new uses for starch, and for materials based on starch, within the broader context of the increasing demand for materials based on renewable resources, will certainly increase the demand for starch production and hence the development of new commercial sources of starch.

15.3 STRUCTURE OF STARCH GRANULES

Starch can be found in various parts of a plant, such as the endosperm, the root, the leaf and the fruit pulp. It is deposited in the form of semi-crystalline granules which are insoluble in cold water and resemble spherulites [13] alternating amorphous and crystalline (or semi-crystalline) lamellae. Native starch is composed of two main macromolecular components, namely amylose and amylopectin [14–21]. The monomer units of these natural polymers are α-D-glucopyranosyl moieties linked by (1 → 4) and (1 → 6) bonds [22]. Amylose is a predominantly α-(1 → 4)-D-glucopyranosyl linear macromolecule. Amylopectin is a highly branched and high molecular weight macromolecule composed mostly of α-(1 → 4)-D-glucopyranose units, with α-(1 → 6)-linkages at intervals of approximately 20 units (Fig. 15.1) [15, 23].

Table 15.1

World production of the main starch crops in 2005 (1 × 1 000 metric tons), (Source FAO, 2005 [11])

Crops	World production in 2005 (1 × 1 000 metric tons)
Maize	711 762.87
Rice (Paddy)	621 588.53
Wheat	630 556.61
Potatoes	324 491.14
Cassava	213 024.81
Bananas	74 236.88
Yams	48 891.21
Sorghum	59 722.09

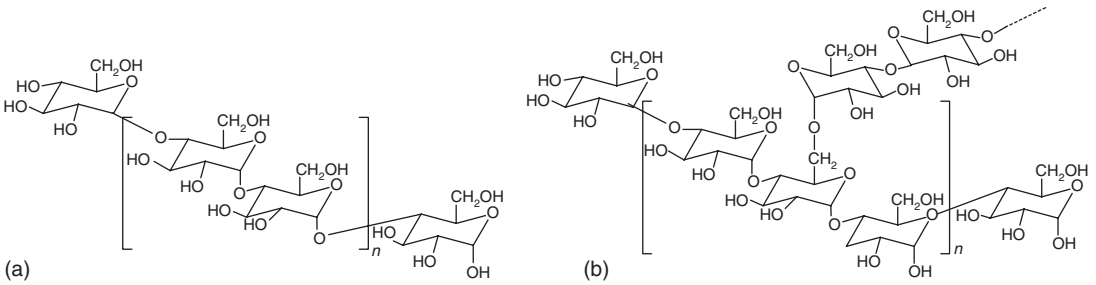

Figure 15.1 Amylose (a) and amylopectin (b) structures.

15.3.1 Granule structure

The starch granule morphology, as well as the structure of its main macromolecular constituents, have been the focus of intense research which is still ongoing because of the complexity of the problems involved. The granules have been examined using several techniques, like light and electron microscopy, X-ray and neutron scattering and more recently, atomic force microscopy [24–28]. Starch granules from different plant species are significantly different and can be, in the majority of cases, identified by light microscopy inspection. The most obvious differences in starch granules are in their shape and size which can vary considerably [1, 29, 30], as reported in Table 15.2 for some common granules.

The morphology of the starch granule varies not only according to the source plant, but also to the different parts of the same plant [16]. Other important factors influencing these aspects are the degree of polymerization (DP) of amylose and amylopectin and the possible presence of other components in the granule such as lipids, proteins and inorganic compounds [18].

The semi-crystalline nature of starch is well known and its most important feature is the alternation of long-range molecular order and amorphous regions, defining the corresponding alternation of crystalline and amorphous lamellae [14, 16, 20, 31].

An idealized structure for the starch granule was proposed recently by Gallant *et al.* [21]. This model describes the crystalline and amorphous amylopectin lamellae into effectively spherical blocklets whose diameter ranges from 20 to 500 nm. These authors also propose the existence of short radial channels of amorphous material. The granule grows alternating radial amorphous and semi-crystalline rings from the *hilum*, forming a lamellar structure. The amylopectin, which comprises around 75 per cent of the granule, is mainly responsible for the granule crystallinity. The crystalline and semi-crystalline lamellae are composed of amylopectin blocklets, forming a crystalline hard shell composed of large blocklets and a semi-crystalline soft shell composed of small blocklets. The crystalline lamellae are around 9–10 nm thick on an average and consist of ordered double-helical amylopectin side chain clusters, interfacing with the more amorphous lamellae of amylopectin branching regions. The size of the semi-crystalline soft-shell blocklets ranges from 20 to 50 nm. Figure 15.2 depicts this structure as proposed by Gallant *et al*.

Table 15.2

Size, shape and amylose content of some starch granules [2, 12, 17, 29, 30]

Source	Diameter (μm)	Amylose content (wt%)	Shape
Maize	5–25	28	Polyhedric
Waxy maize	5–25	~0	Polyhedric
High-amylose	5–35	55–85	Varied smooth spherical to elongated
Cassava	5–35	16	Semi-spherical
Potato	15–100	20	Ellipsoidal
Wheat	20–22	30	Lenticular, polyhedric
Rice normal	5/3–8	20–30	Polyhedric
Banana	26–35	9–13	Elongated oval with ridges

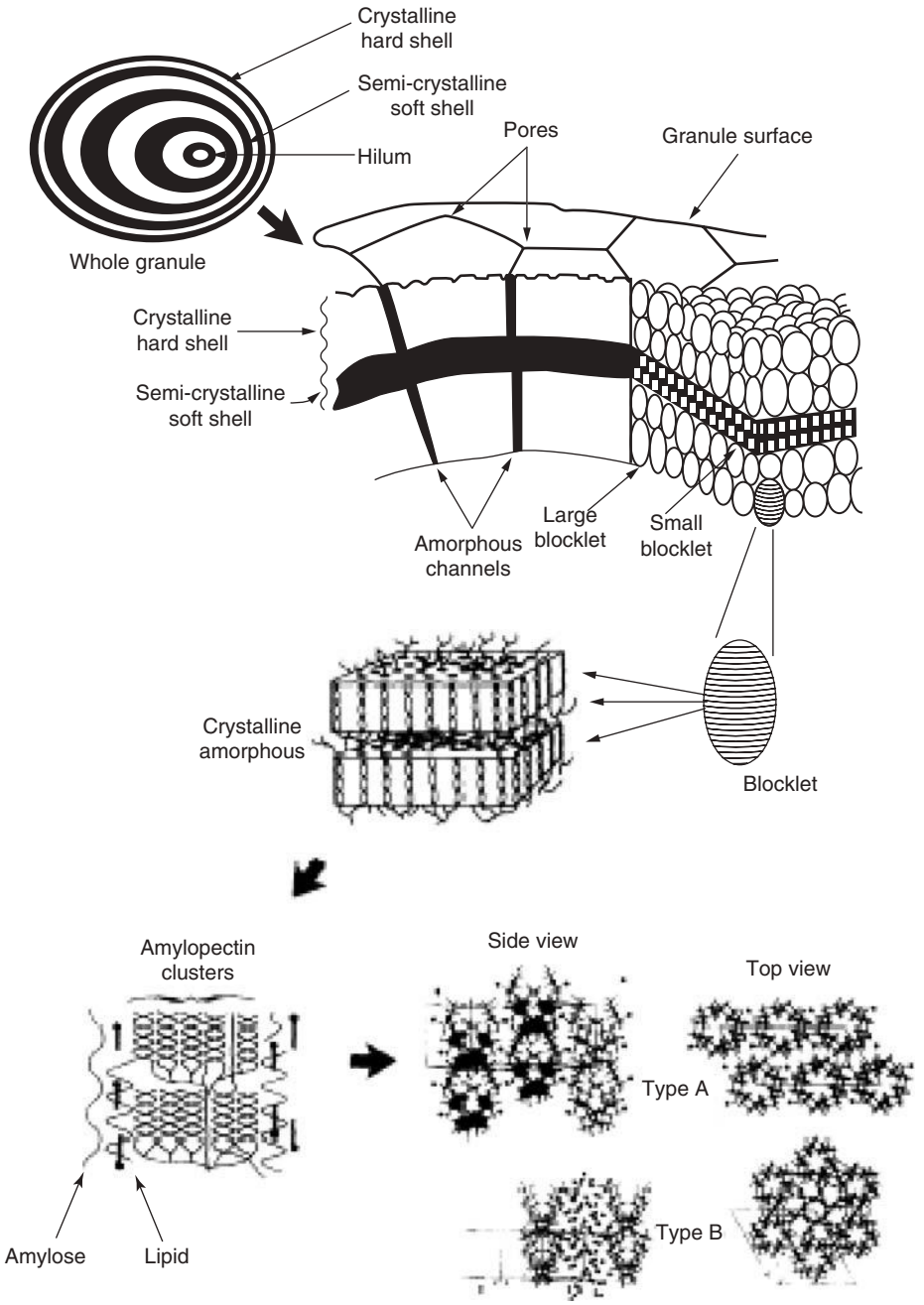

Figure 15.2 Starch granule structure. Reproduced with permission from Reference [21].

15.3.2 Molecular structure and crystallinity

The main technique used to study starch crystallinity is X-ray diffraction, from which starch can be classified to A, B and C crystallites or polymorph forms[17, 32–34]. Starches with these polymorphisms are called A-, B- and C-type, each type presenting its characteristic diffraction patterns. The most commonly observed structures in native starch are A and B, the former being associated mainly with cereal starches, while the latter dominates generally

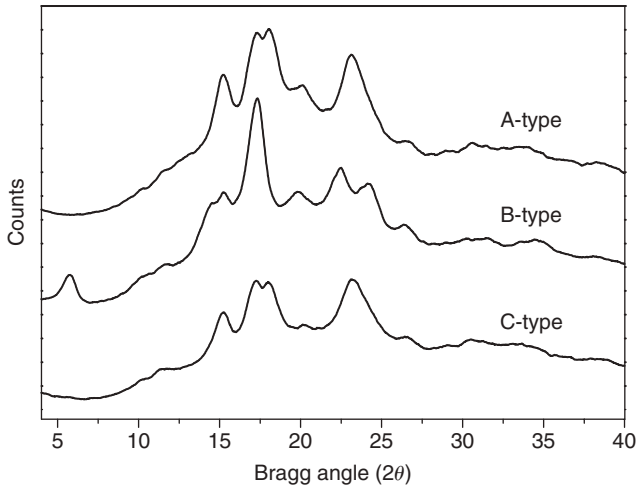

Figure 15.3 X-ray diffraction patterns of maize (A-type), potato (B-type) and cassava (C-type) starches.

in tuber starches but also occurs in maize starches with more than 30–40 per cent amylase [17]. The C pattern is a form intermediate between A and B and is usually associated with pea and various bean starches together with other root starches [34, 35]. A-type crystallites are denser and less hydrated [33] than B^{32}-type counterparts, whereas the C-types arise from the joint presence of the other two homologues [17, 19, 34–40]. Figure 15.3 shows the X-ray diffraction patterns of maize (A-type), potato (B-type) and cassava (C-type) starches.

Although amylose is predominantly made up of linear macromolecules, it has been suggested that amyloses from some starch varieties may contain very long branches [15]. Amylose molecular weights [41] range from 1.5×10^5 to 2.6×10^6, with some variability as a function of the plant variety in the case of cassava [42]. Linear amylose molecules form double helices and can crystallize in a similar way as in starch granule, rendering the A and B structures [15]. Single crystals of low DP amylose in the polymorphisms A and B were also prepared and characterized [43].

Amylose also form complexes with other materials called V-structures (Verkleisterung) [2] which can be isolated in single crystals [43]. The amylose V-complexes are commonly formed by a left-handed amylose helix with six residues per turn enclosing the aliphatic tail of a lipid in its centre [18, 44, 45], each revolution around the lipid taking 8 Å [46, 47]. Depending on the condition of the V-complex formation, they can be crystalline or amorphous [48]. V-complexes are insoluble in water, even in drastic pressure and temperature conditions, a fact that makes them very important in polymer blending when water resistance is a desired property [49, 50].

Amylopectin is a highly branched molecule which is responsible for the main crystalline character of the starch granule. Its structure was modelled as a hyperbranched molecule [17, 51, 52], as proposed initially by Nikuni [53] and French [16] and later improved by Robin [37] (Fig. 15.4). In this model, short chains with 15 D-glucopyranosyl units branch out at almost regular intervals of 25 units to form either external A-chains or internal chains of amylopectin [37]. Starch crystallites are thus formed in compact areas made up of A-chains with DP 15. Less compact areas mainly occupied by B-chains, where the (1,6)-α-D-branching points are located, are placed between these compact areas.

A parallel with synthetic polymers can be made in which amylopectin is a clear cut example of a natural dendrimer [51] with a high degree of branching and a spherical shape, each generation being fully generated from branching sites with a minimum functionality of three.

15.4 DISRUPTION OF STARCH GRANULES

When starch granules are heated in an excess of water (>90 per cent w/w) or of another solvent able to form hydrogen bonding (*e.g.* liquid ammonia, formamide, formic acid, chloroacetic acids and dimethyl sulphoxide) starch undergoes an irreversible order–disorder transition known as gelatinization or destructuration. This

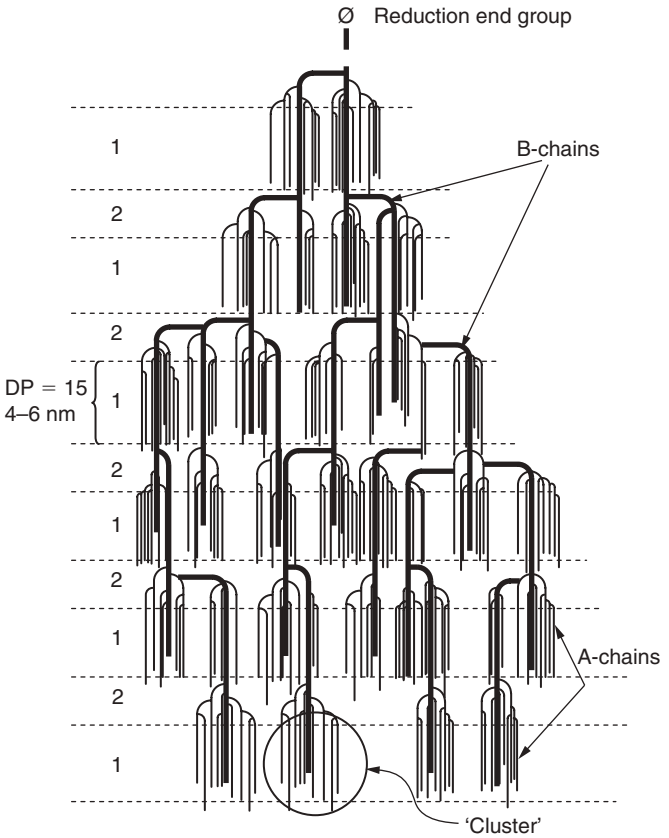

Figure 15.4 Amylopectin model, as proposed in Reference [37]. Reproduced with permission 1 = compact area; 2 = less compact area where the branch points are located, and Ø = reducing moieties.

phenomenon occurs above a characteristic temperature known as the gelatinization temperature. During gelatinization, the amylose is dissolved and progressively leached from the granules. The process can be decomposed into two steps: hydration or diffusion of the solvent through the granule takes place during the first, followed by the melting of the starch crystallites in the second. This process can be studied by a calorimetric technique such as differential scanning calorimetry (DSC) or differential thermal analysis (DTA) [13, 54, 55]. The melting temperature depends on the starch/water ratio. According to Donovan [13], with a large excess of water, only one endotherm appears in the DSC curve, corresponding to the gelatinization temperature, whereas with a modest water content, only one endotherm again appears but at a higher temperature. With intermediate water concentration, two endothermic transitions are observed. This complex behaviour has been treated quantitatively using Flory's relationship between the melting point of the crystalline phases and the quantity of added water [13, 56, 57].

There are several explanations for the multiple peaks in the DSC or DTA curves. One of them is that water is not homogenously distributed in the granule and diffusion can play an important role in that process [13]. This lack of homogeneity leads to partial melting, followed by recrystallization and re-melting, implying that this is a non-reversible process [56] and that the Flory-Huggins theory is not fully applicable to starch gelatinization [13, 56].

Besides DSC and DTA, many other techniques can also be employed to study the order–disorder transition occurring during gelatinization, including X-ray scattering, light scattering, optical microscopy (birefringence using crossed polarizers), thermomechanical analysis and NMR spectroscopy and, more recently, small-angle X-ray scattering (SAXS), wide-angle X-ray scattering (WAXS) and small-angle neutron scattering (SANS) [55]. On the bases of evidence gathered using the latter techniques, Jenkins and Donald [55] concluded that the final loss of crystallinity only occurred when the gelatinization was almost complete.

In an extrusion process, where shear forces and high pressures are applied, the entire process is obviously much more complicated. However, both with limited amounts and excess quantities of water, the main step, associated with the melting of crystallites, is the same and the final mass is an amorphous entanglement of amylose and amylopectin macromolecules.

15.5 APPLICATIONS OF STARCH AS A RAW MATERIAL FOR PLASTIC PRODUCTION

15.5.1 Strategies for the use of starch as a source of polymers

The exploitation of starch as a precursor to macromolecular materials can follow two strategies, *viz.* as a raw material for the production of chemicals used in the synthesis of other polymers or used directly as a high molecular weight polymer by keeping its molecular structure as unchanged as possible.

The first strategy, based on the use of starch for the production of other chemicals was recently reviewed by Robertson *et al.* [58], Koutinas *et al.* [59], Kennedy *et al.* [60] and Otey and Doane [61]. Three different approaches are applied in this context: (i) starch as a raw material for the production of monomers used in the synthesis of polymers which can be non-biodegradable, such as polyethylene, or biodegradable, such as PLA (the main biodegradable commercial polymer whose monomer, lactic acid, can be obtained from the fermentation of starch [62]); (ii) as a raw material for the production of biopolymers like polyhydroxyalkanoates (of which PHB is the main member); (iii) as a raw material for the production of glucose, dextrin and other hydroxyl-containing monomers used in the production of mixed compositions based on starch and other monomers.

In all the above processes, the macromolecular structure of starch is destroyed and the polymers derived from the ensuing monomers are totally different from it. It is important to emphasize that the same monomers (*e.g.* ethylene, sugars and dextrin) can be produced from other sources, both renewable, such as cellulose and sugar cane, and non-renewable such as oil.

The second strategy calls upon the use of starch as such or in combination with other materials and is therefore more interesting than the first one, if anything, in terms of cost and yield. Considering, for example, the conversion of starch into polyethylene through its fermentation to ethyl alcohol and dehydration of the latter, the maximum yield of ethylene produced from starch is close to 35 per cent [61].

In order to adjust the properties of these starch-based materials to the desired application, it is necessary to combine starch with other polymers, as frequently done in the plastic industry. The need for tuneable properties may also require starch modifications, such as esterification or etherification, grafting and reactive or melting extrusion of thermoplastic starch (TPS). The main forms of starch utilization as a polymer are (i) starch grafted with vinyl monomers, (ii) starch as a filler of other polymers and (iii) plasticized starch (PLS), commonly known as TPS.

Of the two major strategies outlined above, the second constitutes the main subject of this chapter.

15.5.2 Use of starch in plastic production

In the 1960s and 1970s, oxidized starch was used successfully in rubber and other polymer formulations [63, 64] and several technologies were developed to optimize its combination with plasticized polyvinyl chloride (PVC) [61, 65, 66].

In 1972, Griffin [66] described the use of starch as particulate filler for low density polyethylene (LDPE) with the aim of giving a paper-like texture and appearance to extrusion-blown LDPE films. The necessity for highly dried starch to avoid defects caused by water volatilization was a financial limitation to the process since it required appropriate storage of the dried starch prior to its use. Another problem was the poor adhesion of the hydrophilic starch granules to the highly hydrophobic polyethylene, which was improved by treating starch with reactive silanes, but this added an extra cost to the process.

In the 1970s and 1980s, the pollution caused by plastic packages considered 'indestructible' become a serious issue and discussions about possible solutions started based on the search for materials which can degrade in landfills, hence research on biodegradable polymers became an important topic.

LDPE–starch blends seemed an interesting approach, but starch granules were encapsulated into the LDPE matrix and thus became, in principle, inaccessible to biodegradation. Later studies carried out by Griffin demonstrated that even when encapsulated, starch could be degraded in an appropriate environment [66]. A further development called upon the use of a pro-oxidant ($Fe3^{+}$, $Mn3^{+}$) in the LDPE matrix [67].

Otey *et al*. [61, 68–71] also described blends of starch with synthetic polymers, in which gelatinized starch was used instead of starch granules. The initial films, composed of 90 per cent of starch blended with poly(ethylene-*co*-acrylic acid) (EAA), were obtained by casting from aqueous dispersions or by dry milling in a rubber mill, followed by a hot roll treatment, but only the cast films attained acceptable characteristics [71]. These materials were intended for application as mulch films for agriculture.

The next generation consisted in compositions of starch and poly(EAA) films, produced by extrusion-blowing [70]. The starch concentration varied between 10 and 40 per cent, that of EAA between 10 and 90 per cent, with some composition also containing between 0 and 50 per cent of PE. Sorbitol and glycerol were also added as plasticizers to some of these mixtures. In a further investigation, Otey *et al*. described a similar composition to which urea, starch-based polyols or glycerol, or mixtures of these materials, were added [72]. Urea was used to improve the gelatinization of starch at lower levels of moisture and the polyols were added to increase the levels of biodegradable materials in the final mixture. A typical composition comprised 40 per cent of starch, 20 per cent of EAA, 15 per cent of urea and 25 per cent of LDPE. These materials were extrusion-blown into films for mulch applications. The critical point for this application is the balance between biodegradability and resistance to it, since the film can neither disintegrate before its estimated lifetime, nor can it offer excessive resistance to biodegradation.

In the 1990s, compositions of starch processed directly in melting equipment such as extruders, were described as a new material named destructurized starch or TPS [57, 73–75]. This material was patented by the Warner-Lambert Company [76] and was described as being prepared with starch that had been heated to a high enough temperature and for enough time for the melting to occur prior to starch degradation. In this process, the starch processed in extruders contained between 5 and 40 per cent of water. It was also claimed that when starch was heated in a closed volume in appropriate moisture and temperature conditions, it became substantially compatible with hydrophobic thermoplastic synthetic polymers.

Lay *et al.* [76] also described destructurized starch compositions with one or two other polymers which included a whole variety of both natural and synthetic macromolecules.

In another patented process, starch was destructurized in the presence of low molecular weight polymers such as polyethylene–vinyl alcohol (EVOH), EAA, poly-ε-caprolactone and small amounts of moisture and of a high boiling point plasticizer, using a high shear equipment like a twin-screw extruder [77]. From these materials emerged one of the most successful commercial thermoplastic materials based on starch, which took the name of Mater-Bi®.

It is also important to mention materials with high starch content, or based exclusively on starch. The main examples of this family of products are expanded TPS compositions such as those patented by the National Starch & Chemical Investment Corp [78] used in replacement of expanded polystyrene. This was one of the first commercial TPSs placed on the market and is still sold today in growing quantities. Its success is due, not only to the replacement of a synthetic polymer by a biodegradable one, but also to its good performance and because its production cost is lower than that of expanded polystyrene.

The grafting of starch with more than 60 per cent of vinyl or acrylic monomers gave materials which showed excellent mechanical and processing properties, being virtually insoluble in water [61]. However, this process was deemed too expensive and thus limited the use of the ensuing grafted starch.

15.6 THERMOPLASTIC STARCH

15.6.1 Definition and properties

The term TPS describes an amorphous or semi-crystalline material composed of gelatinized or destructurized starch containing one or a mixture of plasticizers. TPS can be repeatedly softened and hardened so that it can be moulded/shaped by the action of heat and shear forces, allowing its processing to be conducted with the techniques commonly used in the plastic industry. The following sections are devoted to a brief description of the basics of starch extrusion and processing and to the more relevant applications of TPS [50, 57, 73–75, 79–81]. TPS or destructurized starch are also known as PLS [82], because of the inevitable presence of non-volatile plasticizers in their composition. TPS is however the predominant term used for these materials.

TPS is generally produced by processing a starch/ plasticizer(s) mixture in an extruder at temperatures between 140°C and 160°C at high pressure and high shear. Batch mixers operating in conditions similar to those of extrusion can also be used.

If the final composition contains only water as the plasticizer, in levels greater than 15–20 per cent, it keeps its thermoplastic properties. However if the processing temperature is higher than 100°C, water volatilizes and the melted material expands. If controlled, this is a desired effect, exploited in the production of expanded starch used in packaging as a shock absorber. A common feature of these processing techniques is the limited amount of plasticizer and the consequent high viscosity of the melt, but TPS can also be processed in the presence of large amounts of water, as in the technology developed by Otey *et al.* [71, 72].

The destructuration temperature profile as demonstrated by Donovan [13] in his classical paper depends on the water content of the sample. Hence, the process in the presence of limited amounts of plasticizer appears to be different from that associated with an excess of it. In general, TPS is produced in the former conditions and the shear forces play a fundamental role in its processing [57].

15.6.2 Plasticizers for TPS

The type and quantity of plasticizer employed determine the preparation/processing conditions and the mechanical and thermal properties of the final material, as discussed in several studies [83–93]. Various authors [83–85] extended existing theories related to the glass transition and the effect of plasticizers on it to the glass transition temperature of TPSs. Thus, Kalichevsy and Blanshard [85] applied Couchman and Karasz's approach despite the difficulties associated with a reliable determination of the glass transition temperature of TPSs. Orford *et al.* [83], on the other hand, estimated the T_g of pure amylose and amylopectin at 500 ± 10 K, by measuring the T_g of a series of monodisperse oligomers of increasing DP. This value is above the thermal degradation temperature of these polymers.

Numerous laboratories have tackled the effect of a series of non-volatile plasticizers, such as glycerol [84, 87, 88, 89, 90, 93, 94], urea [84, 87], fructose [85], xylitol, sorbitol, maltitol [87, 90, 94], glycols (EG, TEG, PG, PEG) [84, 87, 89, 94], ethanolamine [92] and formamide [91]. Several criteria concerning the most appropriate structures for this key role have been put forward [94], although a rough first principle simply predicts that any substance capable of forming hydrogen bonds would be able to plasticize starch [95].

The partial or total replacement of water by non-volatile organic solvents (plasticizer) such as glycerol and sugars leads to an increase in the gelatinization temperature, as showed by Perry and Donald [96] a feature which needs to be considered when processing TPS. The reason for this effect is not completely understood, but Perry and Donald attributed it to two main causes, namely, the higher molecular weight plasticizers are less able to penetrate the starch granule and less able to increase the free volume of the amorphous regions, thus being less effective than water as plasticizers. This effect has alternatively been attributed to a reduction in the activity and the volume fraction of water [97]. However, Perry and Donald [96] showed that even glycerol alone can completely gelatinize starch and induce an increase in the gelatinization temperature of approximately 60°C, compared with the water plasticized counterpart. Tan *et al.* [95] recently studied this effect and concluded that the parameters determining solvent transport through the granule, such as viscosity, diffusion and ingress rate, play an important role in determining the gelatinization temperature. Other solvent properties were also considered relevant, such as molecular size and number of possible hydrogen bonds.

15.6.3 Crystallinity in TPS

The crystalline order observed in the native starch granules is completely destroyed in TPS but, because of the mobility of the starch chains, recrystallization occurs, leading generally to the formation of B-, V- and E-type crystalline structures. B-type crystallinity appears after TPS is stored above its glass transition temperature or at high plasticizer contents [81]. V- and E-types are observed just after extrusion and are generated during processing [34, 98, 99]. V-type structures can be observed in two forms, *viz.* V_a-type (anhydrous) for materials containing low moisture contents and V_h-type (hydrated) for materials containing higher moisture contents. E-type occurs only in low moisture compositions, is unstable and rearranges to V-type when the sample is conditioned at ambient temperature with more than 30 per cent relative humidity [34].

As a consequence of their semi-crystalline character, the mechanical properties of TPSs are characteristic of partially crystalline polymers [81] with B-type crystallinity being the major factor influencing the mechanical behaviour of glycerol-PLS [100]. This recrystallization can also be a problem because after long-term storage TPSs can become rigid and brittle.

Despite the fact that TPS is considered a new material in technological terms, its basic features and processes are in fact the same as those relative to extrusion-cooking starch used in the food industry since the 1960s. This kind of processing is therefore briefly described briefly in the heading, given its importance in the development of TPS.

15.6.4 Extrusion-cooking as the basis for TPS

Extrusion has been applied to pasta processing in the food industry since the mid-1930s, but the low temperatures used (<40°C) were insufficient to cook the extruded material. In the 1960s, extrusions at higher temperatures, sufficient to cook the materials, started, and extrusion-cooking became a process of great importance [34]. This process is conducted in the presence of a limited quantity of water (10–25 per cent) at temperatures that can reach 200°C but with very short residence times, so that starch decomposition is minimized. This treatment is known as high temperature short-time (HTST) [34, 101, 102]. The molten starch shows higher viscosities than common plastics during extrusion, and under isothermal conditions it can be described as a pseudoplastic material [34]. The main structural modification associated with extrusion-cooking is the destruction of the starch granule morphology (destructurized starch). However, the process is much more complex and chemical reactions that lead to depolymerization and/or other degradation also take place. The expansion of the melted starch occurs at the extrusion head because of the fast evaporation of the moisture present in the melted starch. This expansion is also an important factor in determining the properties of the extruded material. The overall process was extensively studied by Mercier and Feillet [101] who investigated such variables as the temperature of extrusion (170–200°C), the moisture content of the product before extrusion, and its amylose content.

Extrusion-cooking can be considered the precursor of the modern technology of TPS, the main principles and the changes occurring in starch being the same [63, 75]. Wiedmann and Strobel [80] proposed that the compounding of TPS is a combination of extrusion-cooking and plastic compounding.

15.6.5 Macromolecular scission and starch degradation during destructuring/plasticization

The changes in molecular weight and its distribution play an important role on the rheological and mechanical properties of starch and have therefore received considerable attention [103–106]. The main factors affecting the molecular weight degradation during TPS preparation and processing are the specific mechanical energy applied [103], the temperature and plasticizer content [101, 104].

Gomez and Aguilera [73] studied the effects of water concentration during the extrusion of maize starch on the properties of the ensuing materials and proposed a model for starch degradation during extrusion. In this model, starch granules are converted into gelatinized starch, then into free polymer chains that, depending on the extrusion conditions, can degrade into dextrinized starch and/or oligosaccharides and sugars. One important conclusion of this work was that, when extruding starch with a moisture level below 20 per cent, a product distinct from gelatinized starch is obtained, since it has been partially dextrinized. Dextrins are dextrorotatory products of partially hydrolyzed starch that can be precipitated with alcohol from their aqueous solution [15].

Willett *et al.* [103] studied the melt rheology and molecular weight degradation of waxy maize (amylopectin) in a co-rotating twin-screw extruder by processing the native starch and re-extruding the destructurized material. The moisture content of the first extrusion was 35 per cent and the product was re-extruded with 18 or 23 per cent of moisture. Starch degradation was evaluated by multi-angle light scattering in dimethyl sulphoxide/water. The weight-average molecular weight decreased moderately with increasing specific mechanical energy, which was considered an important parameter for the prediction of the molecular weight degradation during extrusion.

Myllymäki *et al.* studied the depolymerization of barley starch during its extrusion in the presence of a mixture of water and glycerol [107]. They observed a correlation between the depolymerization of the starch chains and both the water/glycerol concentration and, to a minor extent, the screw speed.

Carvalho *et al.* [108] studied chain degradation in TPS composites of maize starch plasticized with glycerol and reinforced with wood pulp. The product was characterized by high performance size-exclusion chromatography (HPSEC). The matrix (starch/glycerol) and the composites were prepared in an intensive batch mixer at 150–160°C, with glycerol and fibre contents in the range of 30–50 per cent and 5–15 per cent, respectively. The HPSEC curves obtained for different levels of plasticization without fibre are shown in Fig. 15.5.

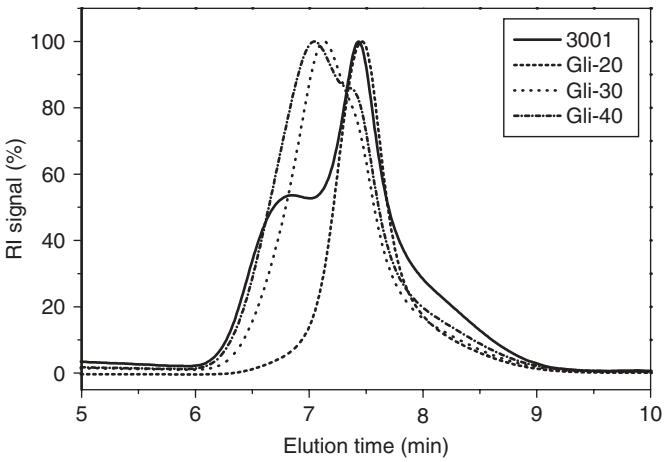

Figure 15.5 High performance size-exclusion chromatography (HPSEC) of native corn starch, and TPS with 20 per cent (Gli-20), 30 per cent (Gli-30) and 40 per cent glycerol (Gli-40). Reproduced with permission from Reference [108].

The results showed that an increase in the glycerol content reduced the starch degradation, whereas an increase in fibre content lead to its increase. The changes in the chromatographic profiles were more pronounced in the high molecular weight fraction, corresponding to amylopectin. The polydispersity index of the matrix was lower than that of native starch due to the selective breakage by shear-induced fragmentation of large amylopectin molecules. The effects of both glycerol and fibres on the molecular weight was of a similar magnitude, in the studied range, for both weight-average molecular weight, M_w, and *z*-weight-average molecular weight, M_z, and these results were expressed in terms of two equations:

$$M_W = 222\,833 + 13\,500G - 13\,000\,F \tag{15.1}$$

$$M_Z = 267\,500 + 19\,250G - 16\,250\,F \tag{15.2}$$

where G and F are normalized glycerol and fibre contents, respectively (30 per cent of glycerol is equal to -1 and 50 per cent of glycerol is equal to $+1$ and likewise for fibres). Glycerol and fibres showed opposite effects, as can be observed in Eq. (15.1 and 15.2). This behaviour is related to the shear-induced fragmentation, *viz.* a process in which the largest molecules are the most susceptible. As this process is highly dependent on the melt viscosity, higher viscosities induce correspondingly higher extents of degradation.

Carvalho *et al.* [109] used ascorbic and citric acid as catalysts for the controlled hydrolytic cleavage of starch macromolecules carried out by melt processing in the presence of glycerol and residual moisture. The process proved effective in providing a means for the controlled tuning of the molecular weight of starch in TPS compositions.

15.6.6 TPS blends

Blending is an important operation for the modification of polymer properties at low cost and without the need of special equipment and techniques. The possibility of blending different polymers increases considerably their range of applications and can also be used to decrease their costs. From a technological point of view, a compatible blend is achieved when the desired properties are improved as a consequence of mixing two or more polymers. In the majority of cases, this mixture is not thermodynamically compatible and constitutes hence a multiphase system [82, 110, 111].

TPS is blended mainly for two purposes. The first, and probably the most important, is to improve such properties as its water resistance and mechanical performances, whereas the second is to use it as a modifier for other polymers with the purpose of increasing the biodegradability and/or decreasing the cost of the ensuing blends. In fact, starch is cheaper than any other polymer, is readily available and renewable, so that major efforts are employed to maximize the starch content in a blend.

Blends of starch with polar polymers containing hydroxyl groups, such as poly(vinyl alcohol), copolymers of ethylene and partially hydrolyzed vinyl acetate have been prepared since the 1970s, as described by Otey *et al.* [61, 68–72]. Since starch and other natural polymers are hydrophilic, water has been commonly used as a plasticizer for these materials. The possibility of using water as plasticizer makes it possible to add the polymer to be blended as an aqueous emulsion, as for example, in the case of natural rubber latex [112], poly(vinyl acetate) and other synthetic polymer lateces [71, 113, 114]. Blends of starch and biodegradable polymers and polymers from renewable resources have been reviewed recently due to their growing importance [82, 110, 111, 115, 116]. Table 15.3 gives some polymers commonly used in blends with starch.

Starch blends can be divided into two main categories according to (1) the source and biodegradation properties of the polymer to be blended with starch and (2) the process used for its preparation. As for the first category, the sources can be obtained directly from renewable resources (biodegradable biopolymers), can be synthetic polymers from either oil or renewable resources, and in this latter case they can be biodegradable or not depending on their structure.

As for the second category, two main processing techniques are used for blending starch, namely melting and solution/dispersion. In *melting processing*, starch blends are obtained during the plasticization of starch granules in an extruder or in a batch mixer. Alternatively, two extruders are used, starch being gelatinized in a first single-screw extruder and then fed into a twin-screw extruder which processes the other component [128, 130]. In *solution or dispersion processing*, the product is often obtained by casting. The first commercial materials produced by solution or dispersion were cast films of starch/EAA blends [61, 71]. A large number of starch blends have been obtained using this procedure, especially when other natural polymers are involved. As in the case of starch, many natural polymers and also biodegradable synthetic polymers are soluble or dispersible in water. Therefore solution/dispersion blending is an interesting option for the production of these mixed materials. In some cases, as for example, when using chitosan, melting is not possible because this polymer decomposes before melting and solution blending is the only viable alternative.

Blending is one of the most promising alternatives to make starch useful as a polymer in replacement of other plastics, and the fast progress occurring in this field is attested by several reviews published recently [82, 110, 111, 115]. Indeed, the commercial plastics based on starch presently available are in the form of blends with other polymers [50, 116].

In TPS blends, starch can be the continuous or the dispersed phase, depending on the starch/second-polymer ratio and on the processing conditions. As a consequence, the interfacial interactions between starch and the other

Table 15.3

Most common polymers used in blending with thermoplastic starch

Polymer	Reference
Poly(vinyl acetate) PVAc	[71, 113]
Poly(methacrylic acid-*co*-methyl methacrylate) MAA/MMA	[114]
Poly(vinyl alcohol) PVA	[119, 120]
Poly(acrylic acid) PAA	[121, 122]
Poly(ethylene-*co*-acrylic acid) EAA	[61, 71]
Poly(ethylene-*co*-vinyl alcohol) EVOH	[49, 50, 123]
Poly(ε-caprolactone) PCL	[50, 124, 125, 123, 109]
Poly(ethylene–vinyl acetate)	[126]
Polyethylene	[127, 128, 129, 126, 130]
Poly(ester–urethanes)	[131]
Poly(*D,L*-lactic acid) PLA	[117, 132, 133]
Poly(3-hydroxybutyrate) PHB	[115]
Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-) PHBV	[125, 134]
Poly(butylene succinate adipate) PBSA	[135]
Polyesteramide PEA	[124, 136]
Zein	[137, 138]
Lignin	[139, 140]
Cellulose and its derivatives	[123]
Natural rubber	[118, 112, 121]

components will determine the properties of the blend. Several approaches have been investigated in order to enhance the compatibility among the components of these blends:

(1) The use of polymers bearing polar groups, particularly those able to form hydrogen bonds (*e.g*. PVA, EAA, EVOH and natural polymers like cellulose and its derivatives, gelatin and zein [49, 50, 110, 115]).
(2) The use of mixtures of polymers where one of them acts as a compatibilizer between starch and less hydrophilic components (*e.g*. PVA in TPS/polyethylene blends or a low molecular weight polymer like poly(ethylene glycol) in TPS/PLA blends [50, 110, 115, 119]).
(3) The use of reactive compatibilizers which can promote a better interface by polymer–polymer chemical interlinking (*e.g*. methylenediphenyl diisocyanate (MDI), pyromellitic anhydride or glycidyl methacrylate [110, 115, 117, 123, 125, 126, 129]).
(4) The formation of complexes between starch and other polymers (*e.g*. V-type complexes [50, 122]).

Arvanitoyannis *et al.* [118] studied blends of starch with 1,4-transpolyisoprene (*gutta percha*) compatibilized with EAA by melt processing and observed that they were biodegradable because of the presence of starch. Their mechanical properties were improved by the addition of glycerol as plasticizer. Rouilly *et al.* [121] also prepared blends of starch and natural rubber by casting mixtures of aqueous starch with glycerol and latex. Carvalho *et al.* [112] blended native starch granules and a natural rubber latex by melt processing calling upon water as a plasticizer for starch. The stable dispersion and the good adhesion between the two natural polymers were attributed in part to the natural non-rubber constituents present in the latex. As little as 2.5 per cent w/w of rubber was sufficient to decrease drastically the brittle character of TPS. Figure 15.6 shows SEM pictures of starch/rubber blends fractured in liquid nitrogen depicting the good dispersion of rubber in the starch matrix.

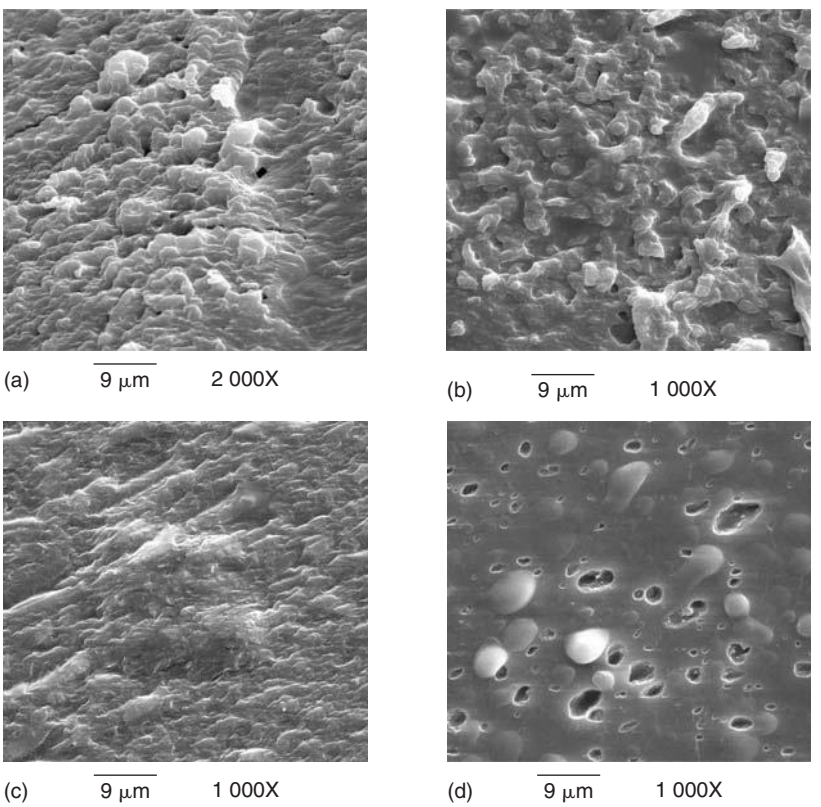

Figure 15.6 Scanning electron micrographs of fragile fractures of starch/natural rubber blends. (a) 20 per cent glycerol and 5 per cent rubber, (b) 30 per cent glycerol and 20 per cent rubber, (c) 40 per cent glycerol and 5 per cent rubber and (d) 40 per cent glycerol and 20 per cent rubber. All quantities are in w/w based on dry matter. Reproduced with permission from Reference [112].

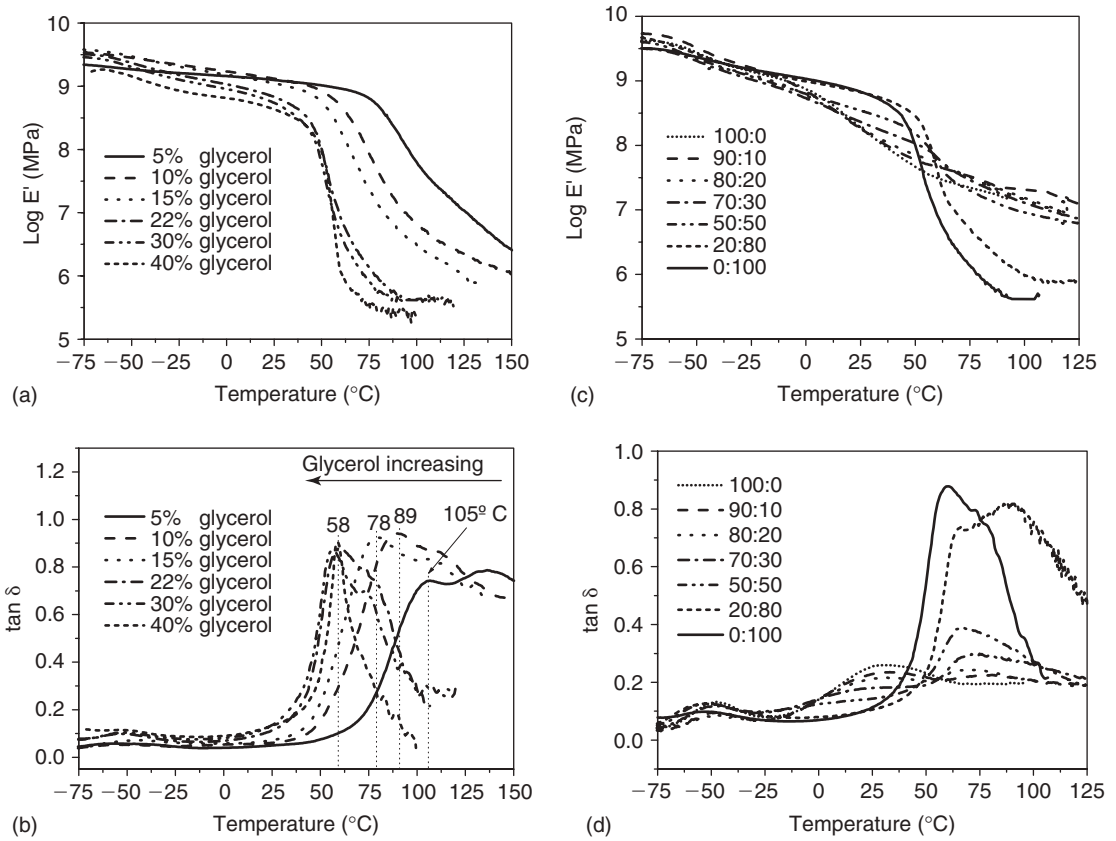

Figure 15.7 Storage modulus (E') and tan δ as a function of temperature for (a–b) plasticized zein with 5, 10, 15, 22, 30 and 40 per cent w/w of glycerol and (c–d) starch/zein blends with 22 per cent w/w of glycerol; the proportions in the legend are starch:zein. Reproduced with permission from Reference [138].

Zein is a protein obtained from maize as a by-product of maize starch production. It is completely amorphous and, despite the fact of being more hydrophobic than starch, it can also be plasticized by glycerol. It is, therefore, an interesting material for use in blends with starch because it shows some compatibility with starch while conferring to the blends a more hydrophobic character [137, 138]. Corradini *et al.* [138] described starch/zein blends prepared by melt processing. The plasticization of zein by glycerol was studied and Figs 15.7(a) and 15.7(b) show the DMA plots of the storage modulus and tan δ as a function of temperature. Above 22 per cent w/w of glycerol, its influence on the glass–rubber transition temperature seized. Consequently, the exceeding plasticizer phase separated and, in the presence of starch, migrated into the TPS-phase. Starch/zein blends are immiscible and, depending on the ratio of these two natural polymers, one or the other generated the continuous phase. Figures 15.7(c) and 15.7(d) show the dynamic mechanical properties of these blends with the two characteristic tan δ peaks corresponding to the starch/glycerol and zein/glycerol phases.

15.6.7 Composites and nanocomposites of TPS

As discussed earlier, TPS has two main drawbacks, namely it is mostly water-soluble and has poor mechanical properties; its reinforcement is one of the available options to overcome these weaknesses. Composite materials in which TPS plays the role of the matrix represent a relatively new topic and hence the literature available is rather modest. Table 15.4 lists some of these reported materials.

Table 15.4

Composites and nanocomposites based on thermoplastic starch

Matrix			Filler/Reinforcement	Reference
Commercial or common name	Description	Source		
Bioplast GS902	Potato starch blends with cellulose derivatives and synthetic polymers	Biotec Emmerich, Germany	Flax, jute, ramie, oil palm fibre	[141]
TPS/PCL-Tone P-787	Wheat starch/40% poly-ε-caprolactone – PCL	Union carbide Antwerp, Belgium	Flax and ramie	[141]
Mater-Bi ZI01U	Blends of corn starch and (poly-ε- caprolactone)	Montedison, Deutchland	Flax and ramie	[141]
TPS/TPU	Blends of TPS/ Thermoplastic polyurethane	Research[a]	Flax	[142]
TPS/PCL	Blends TPS/Poly-ε-caprolactone – PCL	Research[a]	Flax	[142]
Mater Bi	TPS blends with PCL, EVOH, etc. with at least 85% of starch	Novamont/Montedison	Non-woven of flax, hemp, ramie fibres	[143]
SCONACELL A	Modified starch blends	BSL		
Mater-Bi	TPS blends with PCL, etc.	Novamont	Flax cellulose pulp (10–40%)	[144]
TPS/PVA	TPS–polyvinyl alcohol blends	Research	Softowood fiber	[120]
TPS	Starch/Glycerol/Sorbitol–TPS	Research[a]	Regenerated cellulose fibres: Cellunier F and Temming 500	[145]
TPS	Wheat–starch/Glycerol/ Sorbitol–TPS	Research[a]	Flax and ramie	[141]
TPS	Maize starch–TPS	Research[a]	10–20% of flax fibre	[142]
TPS	Starch/Glycerol/ Formamide/Urea	Research[a]	Micro winceyette fiber	[146]
TPS	Starch/Glycerol (30–50% glycerol)	Research[a]	Kraft bleached and thermomechanical wood pulps from *Eucalyptus*	[147, 148]
TPS	Potato starch/Glycerol cast film-TPS	Research[a]	Potatoes microfibrils[b]	[149]
TPS	Waxy maize/Glycerol cast film	Research[a]	Cellulose whiskers (from tunicate)[b]	[150]
TPS	Waxy-maize/Glycerol/ Water cast film	Reseach[a]	starch nanocrystals[b]	[151]
TPS	Wheat starch/Glycerol-TPS	Research[a]	Leafwood fibres	[152]
Mater-Bi	Starch/EVOH	Novamont	Hydroxylapatite-reinforced	[153]
TPS	Maize starch/30% Glycerol	Research[a]	kaolin	[154]
TPS	Potato and wheat starch/∼36.5% Glycerol	Research[a]	Up to 10 wt% of montmorillonite (Closite Na+, Closite 30B-amonium)[b]	[155, 156]

[a] Described in research papers.

[b] Filler or reinforcement with nanometric dimensions.

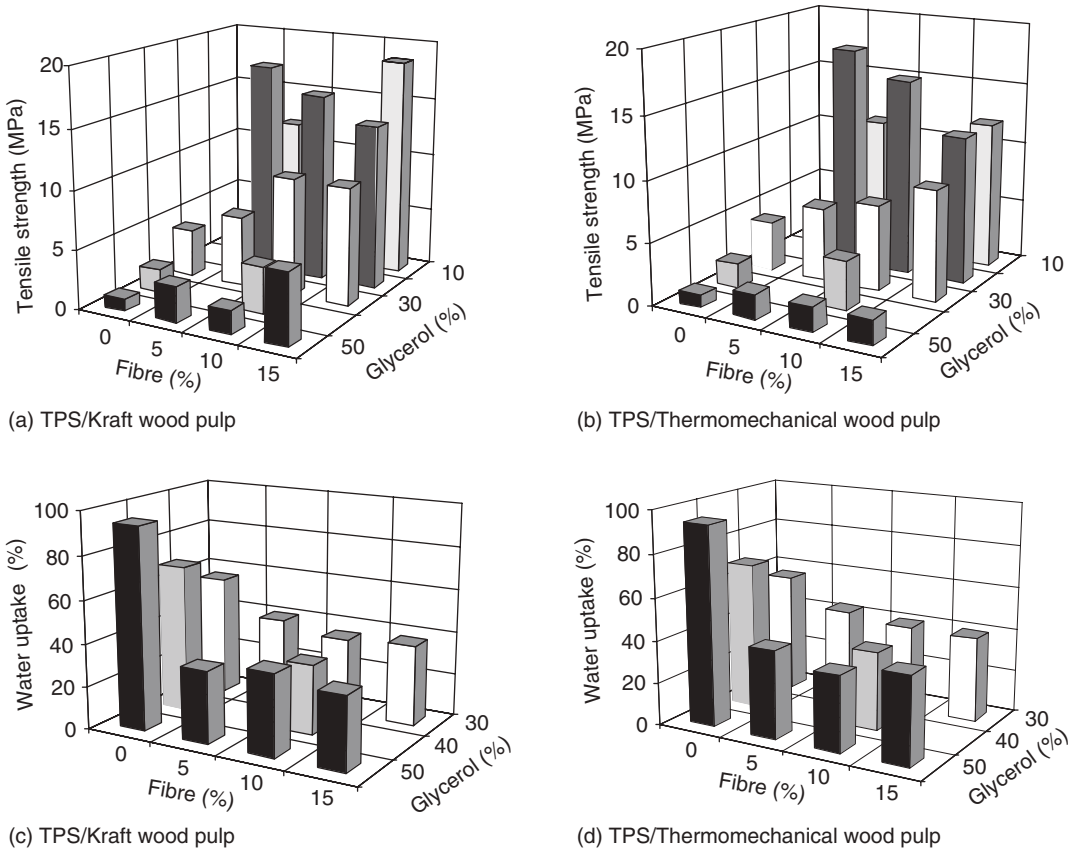

Figure 15.8 Tensile strength and equilibrium water uptake of TPS reinforced with Eucalyptus wood pulps (a–c) Kraft pulp; (b–d) thermomechanical pulp. Reproduced with permission from Reference [147].

Reinforcement has proved to be very effective in improving water resistance, and particularly the mechanical properties of TPS. Increases higher than 100 per cent in tensile strength and higher than 50 per cent in modulus were measured when TPS was reinforced with wood fibres [147–149, 152]. Figure 15.8 shows the notable increase in tensile strength and water uptake from TPS to its composites containing 10–50 wt% of glycerol and 0–15 wt% of Kraft and thermomechanical *Eucalyptus* wood pulp [147, 148]. With the addition of wood fibres, the water uptake at equilibrium decreased considerably, becoming almost independent of both fibre and glycerol contents. This behaviour was attributed mainly to the constraining effect of the reinforcement to the material expansion as water is being absorbed. The effect of lignin, present in thermomechanical fibres, and virtually absent in Kraft counterparts, was negligible.

Kaolin also played a very good role as a reinforcement for TPS matrices as shown by Carvalho *et al*. [154]. The compositions studied in this work were based on TPS containing 30 per cent of glycerol and kaolin in proportions of 10, 20, 30, 40, 50 and 60 phr. Figure 15.9 shows the variation of modulus and tensile strength of these composites as a function of kaolin content with a maximum for both at 50 phr and an increase of 135 and 50 per cent, respectively, relative to the sample prepared without kaolin. The water uptake was reduced considerably for all these kaolin-reinforced TPS composites [154].

A different approach was recently applied to cellulose fibres and starch granules to prepare single-component composites by the partial oxypropylation of these substrates. These novel materials are described in Chapter 12.

15.7 CONCLUSIONS

The necessity to replace materials based on dwindling fossil resources by homologues prepared from renewable counterparts has become an urgent matter for environmental and economical reasons. Within this context, starch

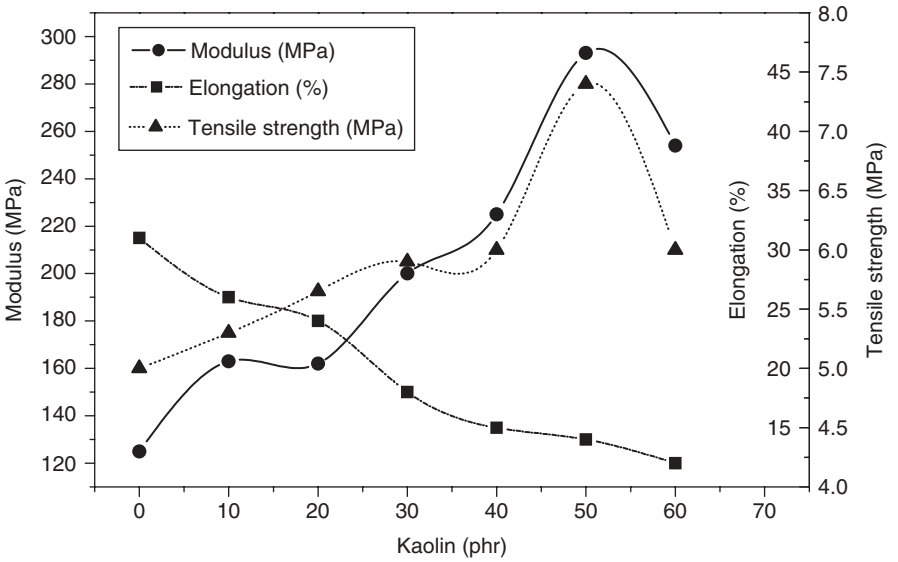

Figure 15.9 Modulus, tensile strength and elongation of TPS/kaolin composites. Reproduced with permission from Reference [154].

will certainly play a very important role as a source of viable alternatives. Its exploitation in non-food applications is not new, but had declined considerably after the Second World War because of the boom of petrochemistry and the development of polymer materials associated with it. The revival of interest in starch-based plastics began at the end of the last millennium with the emergence of new successful commercial products, and is witnessing a vigorous pursuit. The reasons for this success are to be found in the fact that starch is a cheap raw material, readily available ubiquitously (albeit from different species) and very versatile in terms of chemical and physical modifications.

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REFERENCES

1. Jarowenko W., Starch based adhesives, in *Handbook of Adhesives*, Ed.: Skeist I., 2nd Edition, Van Nostrand Reinhold Co., New York, 1977, pp. 192–211, Chapter 12.
2. Daniel J., Whistler R.L., Voragen A.C.J., Pilnik W., Starch and other polysaccharides, in *Ullmann's Encyclopedia of Industrial Chemistry,* Eds.: Elvers B., Hawkins S. and Russey W., 5th Edition, VCH Verlagsgesellschaft mbH, Weinheim, 1994, **Volume A25**, pp. 1–62.
3. Whistler R.L., Bemiller J.N., Paschall E.F., *Starch: Chemistry and Technology*, 2nd Edition, Academic Press, San Diego, CA, 1984, p. 718.
4. Baptist J.N., Process for preparing poly-β-hydroxybutyric acid, US Patent # US 3036959,1962; Baptist J.N., Process for preparing poly-β-hydroxybutyric acid, US Patent # US 3044942, 1962.
5. Baptist J.N., Werber F.X., Plasticized poly-β-hydroxybutyric acid and process, US Patent # US 3182036, 1965.
6. Prescott S.C., Dunn C.G., *Industrial Microbiology*, McGraw-Hill, New York, 1959.
7. Drumright R.E., Gruber P.R., Henton D.E., Polylactic acid technology, *Adv. Mater.*, **12**, 2000, 1841–1846.
8. Tomasik P., Schilling C.H., Chemical Modification of Starch, *Adv. Carbohydr. Chem. Biochem.*, **59**, 2004, 175–403.
9. Galliard T., *Starch: Properties and Potential*, 1st Edition, John Wiley & Sons, New York, 1987. p. 151.
10. Galliard T., Starch availability and utilization, in *Starch: Properties and Potential,* Ed.: Galliard T., 1st Edition, John Wiley & Sons, New York, 1987, pp. 1–15, Chapter 1.

11. FAO (Food and Agriculture Organization of the United Nations), 2007. *FAOSTAT* Statistical database Agriculture, Rome, Italy, data collected on January 2007.
12. Zhang P., Whistler R.L., BeMiller J.N., Hamaker B.R., Banana starch: Production, physicochemical properties, and digestibility – A review, *Carbohydr. Polym.*, **59**, 2005, 443–458.
13. Donovan J.W., Phase transitions of the starch–water system, *Biopolymers*, **18**, 1979, 263–275.
14. Jenkins P.J., Cameron R.E., Donald A.M., A universal feature in the structure of starch granules from different botanical sources, *Starch/Stärke*, **45**, 1993, 417–420.
15. Whistler R.L., Daniel J.R., Molecular structure of starch, in *Starch: Chemistry and Technology*, Eds.: Whistler R.L., Bemiller J.N. and Paschall E.F., 2nd Edition, Academic Press, San Diego, CA, 1984, pp. 153–182, Chapter 6.
16. French D., Organization of starch granules, in *Starch: Chemistry and Technology,* Eds.: Whistler R.L., Bemiller J.N. and Paschall E.F., 2nd Edition, Academic Press, San Diego, CA, 1984, pp. 183–247, Chapter 7.
17. Blanshard J.M.V., Starch granule structure and function: A physicochemical approach, in *Starch: Properties and Potential,* Ed.: Galliard T., 1st Edition, John Wiley & Sons, New York, 1987, pp. 14–54, Chapter 2.
18. Galliard T., Bowler P., Morphology and composition of starch, in *Starch: Properties and Potential*, Ed.: Galliard T., 1st Edition, John Wiley & Sons, New York, 1987, pp. 55–78, Chapter 3.
19. Gidley M.J., Bociek S.M., Molecular organization in starches: A ^{13}C CP/MAS NMR study, *J. Am. Chem. Soc.*, **107**, 1985, 7040–7044.
20. Tang H.R., Godward J., Hills B., The distribution of water in native starch granules – A multinuclear NMR study, *Carbohydr. Polym.*, **43**, 2000, 375–387.
21. Gallant D.J., Bouchet B., Baldwin P.M., Microscopy of starch: Evidence of a new level of granule organization, *Carbohydr. Polym.*, **32**, 1997, 177–191.
22. Kainuma K., Starch oligosaccharides: Linear, branched, and cyclic, in *Starch: Chemistry and Technology*, Eds.: Whistler R.L., Bemiller J.N. and Paschall E.F., 2nd Edition, Academic Press, San Diego, CA, 1984, pp. 125–152, Chapter 5.
23. Cheetham N.W.H., Tao L., Variation in crystalline type with amylose content in maize starch granules: An X-ray powder diffraction study, *Carbohydr. Polym.*, **36**, 1998, 277–284.
24. Ayoub A., Ohtani T., Sugiyama S., Atomic force microscopy investigation of disorder process in rice starch granule surface, *Starch/Stärke*, **58**, 2006, 475–479.
25. Baldwin P.M., Adler J., Davies M.C., Melia C.D., High-resolution imaging of starch granule surface by atomic force microscopy, *J. Cereal Sci.*, **27**, 1998, 255–265.
26. Ohtani T., Yoshino T., Hagiwara S., Maekawa T., High-resolution imaging of starch granule structure using atomic force microscopy, *Starch/Stärke*, **52**, 2000, 153–155.
27. Ridout M.J., Gunning A.P., Parker M.L., Wilson R.H., Morris V.J., Using atomic force microscopy to image the internal structure of starch granules, *Carbohydr. Polym.*, **50**, 2002, 123–132.
28. Ridout M.J., Parker M.L., Hedley C.L., Bogracheva T.Y., Morris V.J., Atomic force microscopy of pea starch: Granule architecture of the rug3-a, rug4-b, rug5-a and lam-c mutants, *Carbohydr. Polym.*, **65**, 2006, 64–74.
29. Snyder E.M., Industrial microscopy of starches, in *Starch: Chemistry and Technology,* Eds.: Whistler R.L., Bemiller J.N. and Paschall E.F., 2nd Edition, Academic Press, San Diego, CA, 1984, pp. 661–673, Chapter 22.
30. Fitt L.E., Snyder E.M., Photomicrographs of starches, in *Starch: Chemistry and Technology*, Eds.: Whistler R.L., Bemiller J.N. and Paschall E.F., 2nd Edition, Academic Press, San Diego, CA, 1984, pp. 675–689, Chapter 23.
31. Cameron R.E., Donald A.M., A small-angle X-ray scattering study of the annealing and gelatinization of starch, *Polymer*, **33**, 1992, 2628–2635.
32. Wu H.C.H., Sarko A., The double-helical molecular structure of crystalline B-amylose, *Carbohydr. Res.*, **61**, 1978, 7–25.
33. Wu H.C.H., Sarko A., The double-helical molecular structure of crystalline A-amylose, *Carbohydr. Res.*, **61**, 1978, 27–40.
34. Colonna P., Buleon A., Mercier C., Physically modified starches, in *Starch: Properties and Potential*, Ed.: Galliard T., 1st Edition, John Wiley & Sons, New York, 1987, pp. 79–115, Chapter 4.
35. Buléon A., Gérard C., Riekel C., Vuong R., Chanzy H., Details of the crystalline ultrastructure of C-starch granules revealed by synchrotron microfocus mapping, *Macromolecules*, **31**, 1998, 6605–6610.
36. Vermeylen R., Goderis B., Reynaers H., Delcour J.A., Amylopectin molecular structure reflected in macromolecular organization of granular starch, *Biomacromolecules*, **5**, 2004, 1775–1786.
37. Robin J.P., Mercier C., Charbonniere R., Guilbot A., Lintnerized starches. Gel filtration and enzymatic studies of soluble residues from prolonged acid treatment if potato starch, *Cereal Chem.*, **51**, 1974, 389–406.
38. Bogracheva T.Y., Morris V.J., Ring S.G., Hedley C.L., The granular structure of C-type pea starch and its role in gelatinization, *Biopolymers*, **45**, 1998, 323–332.
39. Zobel H.F., Starch crystal transformation and their industrial importance, *Starch/Stärke*, **40**, 1988, 1–7.
40. Cairns P., Bogracheva T.Y., Ring S.G., Hedley C.L., Morris V.J., Determination of the polymorphic composition of smooth pea starch, *Carbohydr. Polym.*, **32**, 1997, 275–282.

41. Young A.H., Fractionation of starch, in *Starch: Chemistry and Technology*, Eds.: Whistler R.L., Bemiller J.N. and Paschall E.F., 2nd Edition, Academic Press, San Diego, CA, 1984, pp. 249–283, Chapter 8.
42. Charoenkul N., Uttapap D., Pathipanawat W., Takeda Y., Molecular structure of starches form cassava varieties having different cooked root textures, *Starch/Stärke*, **58**, 2006, 443–452.
43. Buléon A., Duprat F., Single crystals of amylose with low degree of polymerization, *Carbohydr. Polym.*, **4**, 1984, 161–173.
44. Becker A., Hill S.E., Mitchell J.R., Relevance of amylose–lipid complexes to the behaviour of thermally processed starches, *Starch/Stärke*, **53**, 2001, 121–130.
45. Karkalas J., Ma S., Morrison W.R., Pethrick R.A., Some factors determining the thermal properties of amylose inclusion complexes with fatty acids, *Carbohydr. Res.*, **268**, 1995, 233–247.
46. Takeo K., Kuge T., Complexes of starchy materials with organic compounds .3. X-ray studies on amylose and cyclodextrin complexes, *Agric. Biol. Chem.*, **33**, 1969, 1174–1180.
47. Gidley M.J., Bociek S.M., ^{13}C CP/MAS NMR studies of amylose inclusion complexes, cyclodextrins, and the amorphous phase of starch granules: Relationships between glycosidic linkage conformation and solid-state ^{13}C chemical shifts, *J. Am. Chem. Soc*, **110**, 1988, 3820–3829.
48. Godet M.C., Bouchet B., Colonna P., Gallant D.J., Buléon A., Crystalline amylose–fatty acid complexes: Morphology and crystal thickness, *J. Food Sci.*, 1996, 1196–2101.
49. Simmons S., Thomas E.L., Structural characteristics of biodegradable thermoplastic starch/poly(ethylene vinyl alcohol) blends, *J. Appl. Polym. Sci.*, **58**, 1995, 2259–2285.
50. Bastioli C., Properties and applications of Mater-Bi starch-based materials, *Polym. Degrad. Stab.*, **59**, 1998, 263–272.
51. Matheson N.K., Caldwell R.A., α(1–4) Glucan chain disposition in models of α(1–4)(1–6) glucans: Comparison with structural data for mammalian glycogen and waxy amylopectin, *Carbohydr. Polym.*, **40**, 1999, 191–209.
52. Thompson D.B., On the non-random nature of amylopectin branching, *Carbohydr. Polym.*, **43**, 2000, 223–239.
53. Nikuni Z., Studies on starch granules, *Starch/Stärke*, **30**, 1978, 105–111.
54. Liu H., Lelievre J., Ayoung-Chee W.A., A study of starch gelatinization using differential scanning calorimetry, X-ray, and birefringence measurements, *Carbohydr. Res.*, **210**, 1991, 79–87.
55. Jenkins P.J., Donald A.M., Gelatinization of starch: A combined SAXS/WAXS/DSC and SANS study, *Carbohydr. Res.*, **308**, 1998, 133–147.
56. Biliaderis C.G., Page C.M., Maurice T.J., Juliano B.O., Thermal characterization of rice starches: A polymeric approach to phase transitions of granular starch, *J. Agric. Food Chem.*, **34**, 1986, 6–14.
57. Kokini J.L., Lai L.S., Chedid L.L., Effects of starch structure on starch rheological properties, *Food Technol.*, **46**, 1992, 124–139.
58. Robertson G.H., Wong D.W.S., Lee C.C., Wagschal K.W., Smith M.R., Orts W.J., Native or raw starch digestion: A key step in energy efficient biorefining of grain, *J. Agric. and Food Chem.*, **54**, 2006, 353–365.
59. Koutinas A.A., Wang R., Webb C., Evaluation of wheat as generic feedstock for chemical production, *Ind. Crops and Prod.*, **20**, 2004, 75–88.
60. Kennedy J.F., Cabral J.M.S., Sá-Correia I., White C.A., Starch biomass: A chemical feedstock for enzyme and fermentation processes, in *Starch: Properties and Potential*, Ed.: Galliard T., 1st Edition, John Wiley & Sons, New York, 1987, pp. 115–148, Chapter 5.
61. Otey F.H., Doane W.M., Chemicals from starch, in *Starch: Chemistry and Technology,* Eds.: Whistler R.L., Bemiller J.N. and Paschall E.F., 2nd Edition, Academic Press, San Diego, CA, 1984, pp. 389–416, Chapter 11.
62. Garlotta D., A literature review of poly(lactic acid), *J. Polym. Environ.*, **9**, 2001, 63–84.
63. Griffin G.J.L., Gelatinized starch products, in *Chemistry and Technology of Biodegradable Polymers,* Ed.: Griffin G.J.L., 1st Edition, Blackie Academic & Professional, London, 1984, pp. 135–150, Chapter 7.
64. Pfeifer V.F., Sohns V.E., Conway H.F., Lancaster E.B., Dabic S., Griffin E.L., 2-Stage process for dialdehyde starch using electrolytic regeneration of periodic acid, *Ind. Eng. Chem.*, **52**, 1960, 201–206.
65. Westhoff R.P., Otey F.H., Mehltretter C.L., Russell C.R., Starch-filled polyvinyl chloride plastics – Preparation and evaluation, *Ind. Eng. Chem. Prod. Res. Dev.*, **13**, 1974, 123–125.
66. Griffin G.J.L., Particulate starch based products, in *Chemistry and Technology of Biodegradable Polymers,* Ed.: Griffin G. J.L., 1st Edition, Blackie Academic & Professional, London, 1984, pp. 18–47, Chapter 3.
67. Arnaud R., Dabin P., Lemaire J., Al-Malaika S., Chohan S., Coke M., Scott G., Fauve A., Maaroufi A., Photooxidation and biodegradation of commercial photodegradable polyethylenes, *Polym. Degrad. Stab.*, **46**, 1994, 211–224.
68. Otey F.H., Westhoff R.P., Biodegradable film compositions prepared from starch and copolymers of ethylene and acrylic acid, US Patent # US 4133784, January 9, 1979.
69. Otey F.H., Westhoff R.P., Biodegradable starch-based blown films, US Patent # US 4337181, June 29, 1982.
70. Otey F.H., Westhoff R.P., Doane W.M., Title: Starch-based blown films, *Ind. Eng. Chem. Prod. Res. Dev.*, **19**, 1980, 592–595.

71. Otey F.H., Westhoff R.P., Russell C.R., Biodegradable films from starch and ethylene–acrylic acid copolymer, *Ind. Eng. Chem. Prod. Res. Dev.*, **16**, 1977, 305–308.
72. Otey F.H., Westhoff R.P., Doane W.M., Starch-based blown films .2., *Ind. Eng. Chem. Res.*, **26**, 1987, 1659–1663.
73. Gomez M.H., Aguilera J.M., A physicochemical model for extrusion of corn starch, *J. Food. Sci.*, **49**, 1984, 40–49.
74. Röper H., Koch H., The role of starch in biodegradable thermoplastic materials, *Starch/Stärke*, **42**, 1990, 123–130.
75. Shogren R.L., Fanta G.F., Doane W.M., Development of starch based plastics – A reexamination of selected polymer systems in historical perspective, *Starch/Stärke*, **45**, 1993, 276–280.
76. Gustav L., Rehm J., Stepto R.F., Thoma R., Sachetto J-P., Lentz D.J., Silbiger J., Polymer composition containing destructurized starch, US Patent # US 5095054, 1992.
77. Bastioli C., Lombi R., Deltredici G., Guanella I., De-structuring starch for use in biodegradable plastics articles – By heating non-dried, non-water-added starch with plasticizer and enzyme in an extruder, Eur. Pat. # EP400531-A1, 1991.
78. Lacourse N.L., Altieri P.A., Biodegradable shaped products and method of preparation, US Patent # US 5035930, 1991.
79. Lörcks J., Properties and applications of compostable starch-based plastic materials, *Polym. Degrad. Stab.*, **59**, 1998, 245–249.
80. Wiedmann W., Strobel E., Compounding of thermoplastic starch with twin-screw extruders, *Starch/Stärke*, **43**, 1991, 138–145.
81. van Soest J.J.G., de Wit D., Vliegenthart F.G., Mechanical properties of thermoplastic waxy maize starch, *J. Appl. Polm. Sci.*, **61**, 1996, 1927–1937.
82. Avérous L., Biodegradable multiphase systems based on plasticized starch: A review, *J. Macromol. Sci., Part-C.*, **C44**, 2004, 231–274.
83. Orford P.D., Parker R., Ring S.G., Smith A.C., Effect of water as a diluent on the glass transition behavior of malto-oligosaccharides, amylase and amylopectin, *Int. J. Biol. Macromol.*, **11**, 1989, 91–96.
84. Shogren R.L., Swanson C.L., Thompson A.R., Extrudates of cornstarch with urea and glycols: Structure/mechanical property relations, *Starch/Stärke*, **44**, 1992, 335–338.
85. Kalichevsky M.T., Blanshard J.M.V., The effect of fructose and water on the glass transition of amylopectin, *Carbohydr. Polym.*, **20**, 1993, 107–113.
86. Poutanen K., Forsell P., Modification of starch properties with plasticizers, *Trends Polym. Sci.*, **4**, 1996, 128–132.
87. Lourdin D., Bizot H., Colonna P., "Antiplasticization" in starch–glycerol films?, *J. Appl. Polym. Sci.*, **64**, 1997, 1047–1053.
88. Lourdin D., Coignard L., Bizot H., Colonna P., *Polymer*, **38**, 1997, 5401–5406.
89. Hulleman S.H.D., Janssen F.H.P., Feil H., The role of water during plasticization of native starches, *Polymer*, **39**, 1998, 2043–2048.
90. Mathew A.P., Dufresne A., Plasticized waxy maize starch: Effect of polyols and relative humidity on material properties, *Biomacromolecules*, **3**, 2002, 1101–1108.
91. Ma X., Yu J., Formamide as the plasticizer for thermoplastic starch, *J. Appl. Polym Sci.*, **93**, 2004, 1769–1773.
92. Huang M., Yu J., Ma X., Ethanolamine as a novel plasticizer for thermoplastic starch, *Polym. Degrad. Stab.*, **90**, 2005, 501–507.
93. Teixeira E.D., Da Roz A.L., de Carvalho A.J.F., Curvelo A.A.S., Preparation and characterisation of thermoplastic starches from cassava starch cassava root and cassava bagasse, *Macromol. Symposia.*, **229**, 2005, 266–275.
94. Da Roz A.L., Carvalho A.J.F., Gandini A., Curvelo A.A.S., The effect of plasticizers on thermoplastic starch compositions obtained by melt processing, *Carbohydr. Poly.*, **63**, 2006, 417–424.
95. Tan I., Wee C.C., Sopade P.A., Halley P.J., Investigation of the starch gelatinization phenomena in water–glycerol system: Application of modulate temperature differential scanning calorimetry, *Carbohyd. Polym.*, **58**, 2004, 191–204.
96. Perry P.A., Donald A.M., The role of plasticization in starch granule assembly, *Biomacromolecules*, **1**, 2000, 424–432.
97. Derby R.I., Miller B.S., Miller B.H., Trimbo H.B., Visual observation of wheat starch gelatinization in limited water systems, *Cereal Chem.*, **52**, 1975, 702–713.
98. Mercier C., Charbonniere R., Grebaut J. *et al*, Formation of amylose–lipid complexes by twin-screw extrusion cooking of manioc starch, *Cereal Chem.*, **57**, 1980, 4–9.
99. van Soest J.J.G., Essers P., Influence of amylose–amylopectin ratio on the properties of extrude starch plastics shets, *Pure Appl. Chem.*, **A34**, 1997, 1665–1689.
100. Hulleman .S.H.D., Kalisvaart M.G., Janssen F.H.P., Feil H., Vliegenthart J.F.G., Origins of B-type crystallinity in glycerol-plasticized, compression moulded potato starches, *Carbohydr. Polym.*, **39**, 1999, 351–360.
101. Mercier C., Feillet P., Modification of carbohydrate components by extrusion-cooking of cereal products, *Cereal Chem.*, **52**, 1975, 283–297.
102. Lawton B.T., Henderso G.A., Derlatka E.J., The effect of extruder variables on the gelatinization of corn starch, *Can. J. Chem. Eng.*, **50**, 1972, 168–172.
103. Willett J.L., Millard M.M., Jasberg B.K., Extrusion of waxy maize starch: Melt rheology and molecular weight degradation of amylopectin, *Polymer*, **38**, 1997, 5983–5989.

104. Mercier C., Effect of extrusion-cooking on potato starch using a twin-screw French extruder, *Starke*, **29**, 1977, 48–52.
105. van Soest J.J.G., Benes K., de Wit D., Vliegenthart J.F.G., The influence of starch molecular mass on the properties of extruded thermoplastic starch, *Polymer*, **37**, 1996, 3543–3552.
106. Bastioli C., Bellotti V., Rallis A., Microstructure and melt flow behavior of a starch-based polymer, *Rheol. Acta*, **33**, 1994, 307–316.
107. Myllymäki O., Eerikainen T., Suortti T., Forssele P., Linko P., Poutanen K., Depolymerization of barley starch during extrusion in water glycerol mixtures, *Food Sci. Technol., Lebensm. Wiss. Technol.*, **30**, 1997, 351–358.
108. Carvalho A.J.F., Zambon M.D., Curvelo A.A.S., Gandini A., Size exclusion chromatography characterization of thermoplastic starch composites 1. Influence of plasticizer and fibre content, *Polym. Degrad. Stab.*, **79**, 2003, 133–138.
109. Carvalho A.J.F., Zambon M.D., Curvelo A.A.S., Gandini A., Thermoplastic starch modification during melting processing: Hydrolysis catalyzed by carboxylic acids, *Carbohydr. Polym.*, **62**, 2005, 387–390.
110. Wang X.L., Yang K.K., Wang Y.Z., Properties of starch blends with biodegradable polymers, *J. Macromol. Sci., Part C*, **C43**, 2003, 385–409.
111. Amass W., Amass A., Tighe B., A review of biodegradable polymers: Uses, current development in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polyesters and recent advances in biodegradable studies, *Polym. Int.*, **47**, 1998, 89–144.
112. Carvalho A.J.F., Job A.E., Alves N., Curvelo A.A.S., Gandini A., Thermoplastic starch/natural rubber blends, *Carbohydr. Polym.*, **53**, 2003, 95–99.
113. Ritter H.W., Bergner D.R., Kempf K.W., Starch-based materials and/or molded parts modified by synthetic polymer compounds and process for production the same. US Patent # US 5439953, 1995.
114. Bortnick N.M., Graham R.K., LaFleur E.E., Work W.J., Wu J.C., Melt-processed polymer blends, US Patent # US 5447669, 1995.
115. Yu L., Dean K., Li L., Polymer blends and composites from renewable resources, *Prog. Polym. Sci.*, **31**, 2006, 502–576.
116. Bastioli C., Global status of the production of biobased packaging materials, *Starch/Stärke*, **53**, 2001, 351–355.
117. Wang H., Sun X.Z., Seib P., Mechanical properties of poly(lactic acid) and wheat starch blends with methylenediphenyl diisocyanate, *J. Appl. Polym. Sci.*, **84**, 2002, 1257–1262.
118. Arvanitoyannis I., Kolokuris I., Nakayama A., Aiba S., Preparation and study of novel biodegradable blends based on gelatinized starch and 1,4-trans-polyisoprene (gutta percha) for food packaging or biomedical applications, *Carbohydr. Polym.*, **34**, 1997, 291–302.
119. Follain N., Joly C., Dole P., Roge B., Mathlouthi M., Quaternary starch based blends: Influence of a fourth component addition to the starch/water/glycerol system, *Carbohydr. Polym.*, **63**, 2006, 400–407.
120. Shogren R.L., Lawton J.W., Tiefenbacher K.F., Baked starch foams: Starch modifications and additives improve process parameters, structure and properties, *Ind. Crops. Prod.*, **16**, 2002, 69–79.
121. Rouilly A., Rigal L., Gilbert R.G., Synthesis and properties of composites of starch and chemically modified natural rubber, *Polymer*, **45**, 2004, 7813–7820.
122. Biswas A., Willet J.L., Gordon S.H., Finkenstadt V.L., Cheng H.N., Complexation and blending of starch, poly(acrylic. acid), and poly(N-vinyl pyrrolidone), *Carbohydr. Polym.*, **65**, 2006, 397–403.
123. Demirgöz D., Elvira C., Mano J.F., Cunha A.M., Piskin E., Reis R.L., Chemical modification of starch based biodegradable polymeric blends: Effects on water uptake, degradation behaviour and mechanical properties, *Polym Degrad. Stab.*, **70**, 2000, 161–170.
124. Avérous L., Moro L., Dole P., Fringant C., Properties of thermoplastic blends: Starch–polycaprolactone, *Polymer*, **41**, 2000, 4157–4167.
125. Avella M., Errico M.E., Laurienzo P., Martuscelli E., Raimo M., Rimedio R., Preparation and characterization of compatibilized polycaprolactone/starch composites, *Polymer*, **41**, 2000, 3875–3881.
126. Mani R., Bhattacharya M., Properties of injection moulded starch/synthetic polymers blends-III. Effect of amylopectin to amylose ratio in starch, *Eur. Polym. J.*, **34**, 1998, 1467–1475.
127. Griffin G.J.L., Starch polymer blends, *Polym. Degrad. Stab.*, **45**, 1994, 241–247.
128. St-Pierre N., Favis B.D., Ramsay B.A., Ramsay J.A., Verhoogt H., Processing and characterization of thermoplastic starch/polyethylene blends, *Polymer*, **38**, 1997, 647–655.
129. Bikiaris D., Prinos J., Koutsopoulos K., Vouroutzis N., Pavlidou E., Frangis N., Panayiotou C., LDPE/plasticized starch blends containing PE–g–MA copolymer as compatibilizer, *Polym. Degrad Stab.*, **59**, 1998, 287–291.
130. Rodriguez-Gonzalez F.J., Ramsay B.A., Favis B.D., High performance LDPE/thermoplastic starch blends: A sustainable alternative to pure polyethylene, *Polymer*, **44**, 2003, 1517–1526.
131. Seidenstucker T., Fritz H.G., Innovative biodegradable materials based upon starch and thermoplastic poly(ester-urethane) (TPU), *Polym. Degrad. Stab.*, **59**, 1998, 279–285.
132. Zhang J.F., Sun X.Z., Mechanical properties of poly(lactic acid)/starch composites compatibilized by maleic anhydride, *Biomacromolecules*, **5**, 2004, 1446–1451.

133. Martin O., Avérous L., Poly(lactic acid): Plasticization and properties of biodegradable multiphase systems, *Polymer*, **42**, 2001, 6209–6219.
134. Kotnis M.A., Obrien G.S., Willett J.L., Processing and mechanical-properties of biodegradable poly(hydroxybutyrate-co-valerate)–starch compositions, *J. Env. Polym. Degrad.*, **3**, 1995, 97–105.
135. Avérous L., Fringant C., Association between plasticized starch and polyesters: Processing and performances of injected biodegradable systems, *Polym. Eng. Sci.*, **41**, 2001, 727–734.
136. Schwach E., Avérous L., Starch-based biodegradable blends: Morphology and interface properties, *Polym. Int.*, **53**, 2004, 2115–2124.
137. Chanvrier H., Colonna P., Della Valle G., Lourdin D., Structure and mechanical behaviour of corn flour and starch–zein based materials in the glassy state, *Carbohydr. Polym.*, **59**, 2005, 109–119.
138. Corradini E., de Medeiros E.S., Carvalho A.J.F., Curvelo A.A.S., Mattoso L.H.C., Mechanical and morphological characterization of starch/zein blends plasticized with glycerol, *J. Appl. Polym. Sci.*, **101**, 2006, 4133–4139.
139. Baumberger S., Lapierre C., Monties B., Della Valle G., Use of kraft lignin as filler for starch films, *Polym Degrad. Stab.*, **59**, 1998, 273–277.
140. Morais L.C., Curvelo A.A.S., Zambon M.D., Thermoplastic starch–lignosulfonate blends. 1. Factorial planning as a tool for elucidating new data from high performance size-exclusion chromatography and mechanical tests, *Carbohydr. Polym.*, **62**, 2005, 104–112.
141. Wollerdorfer M., Bader H., Influence of natural fibres on the mechanical properties of biodegradable polymers, *Ind. Crops Prod.*, **8**, 1998, 105–112.
142. Mittenzwey R., Seidenstücker T., Fritz H., Süβmuth R., Prüfung der umweltverträglichkeit neu entwickelter polymerwerkstoffe auf der basis nachwachsender rohstoffe durch ein einfaches testsystem, *Starch/Stärke*, **10**, 1998, 438–443.
143. Herrmann A.S., Nickel J., Riedel U., Construction materials based upon biologically renewable resources – from components to finished parts, *Polym. Degrad. Stab.*, **59**, 1998, 251–261.
144. Puglia D., Tomassucci A., Kenny J.M., Processing, properties and stability of biodegradable composites based on Mater-Bi-(R) and cellulose fibres, *Polym. Adv. Technol.*, **14**, 2003, 749–756.
145. Funke U., Bergthaller W., Lindhauer M.G., Processing and characterization of biodegradable products based on starch, *Polym. Degrad. Stab.*, **59**, 1998, 293–296.
146. Ma X.F., Yu J.G., Kennedy J.F., Studies on the properties of natural fibers-reinforced thermoplastic starch composites, *Carbohydr. Polym.*, **62**, 2005, 19–24.
147. Carvalho A.J.F., Curvelo A.A.S., Agnelli J.A.M., Wood pulp reinforced thermoplastic starch composites, *Int. J. Polym. Mater.*, **51**, 2002, 647–660.
148. Curvelo A.A.S., Carvalho A.J.F., Agnelli J.A.M., Thermoplastic starch-cellulosic fibers composites: Preliminary results, *Carbohydr. Polym.*, **45**, 2001, 183–188.
149. Dufresne A., Vignon M.R., Improvement of starch film performance using cellulose microfibrils, *Macromolecules*, **31**, 1998, 2693–2696.
150. Anglès M.N., Dufresne A., Plasticized starch/tunicin whiskers nanocomposites materials. 2. Mechanical behavior, *Macromolecules*, **34**, 2001, 2921–2931.
151. Angellier H., Molina-Boisseau S., Dole P., Dufresne A., Thermoplastic starch–waxy maize starch nanocrystals nanocomposites, *Biomacromolecules*, **7**, 2006, 531–539.
152. Avérous A., Frigant C., Moro L., Plasticized starch–cellulose interactions in polysaccharide composites, *Polymer*, **42**, 2001, 6565–6572.
153. Reis R.L., Cunha A.M., Allan P.S., Bevis M.J., Structure development and control of injection-molded hydroxylapatite-reinforced starch/EVOH composites, *Adv. Polym. Technol.*, **16**, 1997, 263–277.
154. Carvalho A.J.F., Curvelo A.A.S., Agnelli J.A.M., A first insight on composites of thermoplastic starch and kaolin, *Carbohydr. Polym.*, **45**, 2001, 189–194.
155. Park H.M., Lee W.K., Park C.Y., Cho W.J., Ha C.S., Environmentally friendly polymer hybrids, *J Mater. Sci.*, **38**, 2003, 909–915.
156. Bagdi K., Müller P., Pukánszky B., Thermoplastic starch/layered silicate composites: Structure, interactions, properties, *Compos. Interfaces*, **13**, 2006, 1–17.

– 16 –

Cellulose Chemistry: Novel Products and Synthesis Paths

Thomas Heinze and Katrin Petzold

ABSTRACT

Novel paths for homogeneous and regioselective functionalization of cellulose are discussed. The acylation of cellulose can be efficiently carried out by homogeneous phase chemistry applying solvents based on polar aprotic media or in ionic liquids and *in situ* activation of the carboxylic acid. Some unconventional cellulose derivatives are described. The regioselective derivatization of protected cellulosics leading to 3-*O*-, 2,3-*O*-, and 6-*O*-functionalized products is of recent interest showing remarkable differences in properties compared with common cellulose derivatives. The nucleophilic displacement reactions with cellulose tosylates provide a further tool for the design of biopolymer-based structures and properties.

Keywords

Cellulose, Polysaccharide, Activation, Cellulose solvents, Esterification, Etherification, Ionic liquids, NMR spectroscopy, Nucleophile displacement, Protecting groups, Regioselective functionalization

16.1 INTRODUCTION

The chemical modification of polysaccharides is still underestimated regarding the structure and hence property design of biopolymer-based materials. At present, the cellulose derivatives commercially produced on a large scale are limited to some esters with C_2 to C_4 carboxylic acids, including mixed esters and phthalic acid half esters and some ethers with methyl-, hydroxyalkyl-, and carboxymethyl functions. In general, the organic chemistry of cellulose opens the way to a broad variety of products by esterification and etherification. In addition, novel products may be obtained by nucleophilic displacement reactions, unconventional chemistry like 'click reactions' and controlled oxidation. The aim of this chapter is to highlight selected recent advances in the chemical modification of cellulose for the synthesis of new products and alternative synthetic paths, in particular under homogeneous conditions, that is, starting with the dissolved polymer, with emphasis on the research from the authors' laboratory. Some issues related to cellulose solvents are also discussed.

16.2 CARBOXYLIC ACID ESTERS

The esterification of cellulose with carboxylic acids is among the most versatile transformations leading to a wide variety of valuable products. The commercial production of cellulose esters is carried out exclusively under heterogeneous reaction conditions, at least at the beginning of the conversion, due to the high cost of solvents and the ease of work-up procedures in the case of multiphase conversions. Completely functionalized derivatives are

generally sought, because partial functionalization mainly leads to insoluble or partly soluble products. For a better control of the reaction temperature and a reduction in the amount of catalyst, acetylation is carried out preferably in methylene chloride, which is associated with the dissolution of the products formed in the final phase of the reaction. It should be pointed out that the dissolution of the cellulose acetate formed in the reaction medium must not be assimilated with the homogeneous reactions where the cellulose is initially dissolved in a solvent like *N*,*N*-dimethyl acetamide (DMA)/LiCl.

A variety of solvent systems have been established for the homogeneous acylation of cellulose at the laboratory scale. These homogeneous reactions permit the synthesis of highly soluble polymers, partially derivatized polymers and are the prerequisite for the application of the 'state of the art' organic reagents yielding a broad structural diversity [1]. The scope of this section is to focus on homogeneous reactions starting with the dissolved cellulose by applying novel activation procedures for the carboxylic acids.

16.2.1 Cellulose solvents

The basic requirement for cellulose dissolution is that the solvent is capable of interacting with the hydroxyl groups of the AGU, so as to eliminate, at least partially, the strong intermolecular hydrogen bonding between the polymer chains. There are two basic schemes for cellulose dissolution:

(1) where it results from physical interactions between cellulose and the solvent;
(2) where it is achieved via chemical reaction leading to covalent bond formation, these solvents usually being called 'derivatizing solvents'.

Route (1) is addressed in detail below because the 'real' solvents are mostly used as the reaction medium for the introduction of unconventional functional groups onto cellulose.

16.2.1.1 Derivatizing solvents

Certain solvents react with cellulose leading to a disruption of the hydrogen bonding by a combination of steric interactions and decrease of the number of hydroxyl groups available for hydrogen bonding. The covalent bond formed should be easily cleavable, for example, by hydrolysis or a change in the pH value of the system. Examples of derivatizing solvents, including the intermediate formed, are summarized in Fig. 16.1 [2]. One problem of this approach of cellulose dissolution is its poor reproducibility due to side reactions and the formation of undefined structures. Nevertheless, various esterification reactions were successfully carried out in these media [2].

The importance of this scheme is that it may lead to an *inverse pattern of functionalization*, provided that special reaction conditions are employed, namely, the involvement of the secondary hydroxyl groups, due to the fact that the primary counterparts that is the more reactive sites, are blocked by the moiety (nitrite, methylol, or reactive acyl function) introduced during dissolution. In some instances, the intermediate formed prior to the onset of conversion (under non-aqueous conditions) into the final product was isolated and dissolved in a common organic solvent, for example dimethyl sulfoxide (DMSO) and *N*,*N*-dimethyl formamide (DMF), to achieve the actual functionalization. At present, this synthesis path is not considered as a viable tool for esterification, although it gives access to novel products.

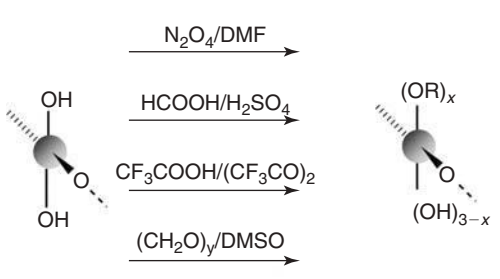


Figure 16.1 Typical derivating solvents for cellulose.

16.2.1.2 Non-derivatizing solvents

The treatment of cellulose with polar solvents capable of interacting with the hydroxyl groups of the polymer may achieve activation/dissolution in a single step. Ideally, the solvent, either mono- or poly-component, should be able to dissolve the cellulose of varying degree of polymerization (DP) and crystallinity without extensive degradation. In addition to the solution stability, the solvent should be dipolar in order to stabilize the highly polar complex of the acyl-transfer, but should not compete with cellulose for the derivatizing agent. To guarantee a complete homogeneous reaction, cellulose and all intermediate states, as well as the final product, should be soluble.

Alkali hydroxide solutions possess some of these requirements and dissolve the biopolymer of rather low DP after swelling in aqueous 8–9 per cent NaOH, subsequently freezing, thawing, and then diluting to a 5 per cent alkali concentration. Urea enhances the solubility due to the fact that it is able to weaken intermolecular hydrogen bonds [3]. However, the base will consume most of the esterifying agent before it reacts with the cellulose and therefore these systems cannot be employed in this context.

The aqueous systems Ni(tren) and Cd(tren) [tren = tris(2-aminoethyl)amine] dissolve cellulose by coordinating with the secondary hydroxyl groups at C2 and C3 [4]. Several molten salt hydrates, for example $LiClO_4 \times 3H_2O$, $LiX \times nH_2O$ ($X = I^-$, NO_3^-, $CH_3CO_2^-$, ClO_4^-), dissolve even high DP cellulose. Because these solvents contain water, which will compete with the cellulose for the derivatizing agent, they have also found limited interest in esterification reactions.

From the synthetic point of view, more flexibility is achieved by employing non-aqueous non-derivatizing solvents. Extensive work has been carried out on binary or ternary mixtures like inorganic or organic electrolytes in strongly dipolar aprotic solvents. The best known example is LiCl in DMA, in *N*-methyl-2-pyrrolidone (NMP), or in 1,3-dimethyl-2-imidazolidinone (DMI) [5]. The structure of these cellulose/solvent system complexes has been described by several authors, differing essentially in the role played by the Li^+ and Cl^- ions, as comprehensively discussed in a specific review [6].

Quite recent work by one of the authors showed that the combination of tetra-*n*-butylammonium fluoride trihydrate ($TBAF \times 3H_2O$) and DMSO was an efficient system capable of dissolving cellulose with a DP as high as 650 within some minutes, without any pretreatment [7]. Cellulose of higher DP was also solubilized, although, in this case, a pretreatment was necessary. A mixture of benzyltrimethylammonium fluoride monohydrate ($BTMAF \times H_2O$) and DMSO displayed a lower dissolving power [8]. Using these new solvents based on DMSO/F^- for the homogeneous acylation of cellulose, the values of the degree of substitution (DS) of the products are influenced by the medium water content [9]. Consequently, the use of water-free TBAF is of interest to understand the solubility and reactivity of cellulose dissolved in DMSO/TBAF. The dehydration of $TBAF \times 3H_2O$, resulting in the water-free salt, is impossible because anhydrous TBAF is unstable, undergoing a rapid E2 elimination giving the formation of bifluoride ions [10]. It was recently reported that anhydrous TBAF can be prepared *in situ* by reacting tetrabutylammonium cyanide and hexafluorobenzene in dry DMSO [11]. This freshly prepared DMSO/TBAF solution readily dissolved cellulose, even in the presence of the hexacyanobenzene by-product, for example, the dissolution of bleached cotton fibres (DP = 3 743) occurred within 1 min, as visualized by optical microscopy [8].

There are few single-component solvents for cellulose. Among them, *N*-ethylpyridinium chloride has a melting point of 118–120°C, which can be decreased by the addition of DMSO, DMF, pyridine, and NMP, leading to a value of 75°C [12]. *N*-methylmorpholine-*N*-oxide $\times H_2O$ (NMNO) is an efficient solvent employed in the production of Lyocell fibres [13, 14]. Its use, however, has various shortcomings with regard to cellulose chemistry namely (1) it is rather thermally unstable, becoming dangerous with a water content below the monohydrate, (2) it tolerates only some reagents yielding mostly a highly viscose gel-like state, and (3) its water content will partly consume the acylating reagent [15, 16].

More recently, some ionic liquids (IL) were shown to be good cellulose solvents over a wide range of DP values without covalent interaction, especially those based on substituted imidazolium ions (Table 16.1) [17]. Even bacterial cellulose with a DP of 6 500 was found to dissolve in one of these solvents [18].

16.2.2 Homogeneous acylation

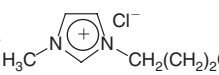
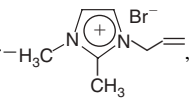
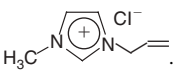
One of the first examples of a completely homogeneous acetylation was the reaction of cellulose with acetic anhydride in *N*-ethylpyridinium chloride in the presence of pyridine, leading to a product with a DS 2.65 within the short reaction time of 44 min [19]. Although the preparation of cellulose triacetate, which is completed within 1 h, needs

Table 16.1

Results of the homogeneous acetylation of cellulose in ionic liquids

Cellulose[a]		Ionic liquid[b]	Reagent		DS[d]	Reference
Type	DP		Type	Mol/mol		
MC	290	$[C_4mim]^+Cl^-$	Acetic anhydride	3	1.87	[22]
MC	290	$[C_4mim]^+Cl^-$	Acetic anhydride	5	2.72	[22]
MC	290	$[C_4mim]^+Cl^-$	Acetic anhydride	10[c]	3.0	[22]
MC	290	$[C_4mim]^+Cl^-$	Acetyl chloride	3	2.81	[22]
MC	290	$[C_4mim]^+Cl^-$	Acetyl chloride	5	3.0	[22]
MC	290	$[C_2mim]^+Cl^-$	Acetic anhydride	3	3.0	[23]
MC	290	$[C_4dmim]^+Cl^-$	Acetic anhydride	3	2.92	[23]
MC	290	$[Admim]^+Br^-$	Acetic anhydride	3	2.67	[23]
SSP	590	$[C_4mim]^+Cl^-$	Acetyl chloride	3	3.0	[22]
SSP	590	$[C_4mim]^+Cl^-$	Acetyl chloride	5	3.0	[22]
CL	1 200	$[C_4mim]^+Cl^-$	Acetyl chloride	3	2.85	[22]
CL	1 200	$[C_4mim]^+Cl^-$	Acetyl chloride	5	3.0	[22]
BC	6 500	$[C_4mim]^+Cl^-$	Acetic anhydride	3	2.25	[18]
BC	6 500	$[C_4mim]^+Cl^-$	Acetic anhydride	5	2.50	[3]
BC	6 500	$[C_4mim]^+Cl^-$	Acetic anhydride	10	3.0	[18]
DSP	650	$[Amim]^+Cl^-$	Acetic anhydride	3	1.99	[21]
DSP	650	$[Amim]^+Cl^-$	Acetic anhydride	5	2.30	[21]

[a] MC, microcrystalline cellulose; SSP, spruce sulphite pulp; CL, cotton linters; BC, bacterial cellulose; DSP, dissolving pulp.

[b] $[C_4mim]^+Cl^-$  , $[C_2mim]^+Cl^-$  , $[Admim]^+Br^-$  , $[Amim]^+Cl^-$  .

[c] Additionally 2.5 mol pyridine per mol anhydroglucose unit (AGU).

[d] Degree of substitution determined by ^{1}H-NMR spectroscopy after perpropionylation [39].

to be carried out at 85°C, it proceeds for cellulose samples with DP values below 1 000 without degradation, that is strictly polymeranalogous. Cellulose acetate samples with a predefined solubility, for example in water, acetone, or chloroform, respectively, are accessible in one step, in contrast to the heterogeneous conversion. A correlation between solubility and distribution of substituents has been attempted by means of ^{1}H NMR spectroscopy [20].

Ionic liquids are promising new solvents in the field of cellulose shaping and functionalization. The acylation of cellulose dissolved in an ionic liquid can be carried out with acetic anhydride. The reaction succeeds without an additional catalyst. Starting from DS 1.86, the cellulose acetates obtained are acetone soluble [21]. The control of the DS by the prolongation of the reaction time is possible. When acetyl chloride is used, complete acetylation is achieved in 20 min (Table 16.1) [18, 22, 23]. This method may lead to a widely applicable acylation procedure for polysaccharides, if the regeneration of the solvent becomes possible.

DMA/LiCl, widely used in peptide and polyamide chemistry, is among the best studied solvents, because it dissolves cellulose of various DP quite readily [24] and shows almost no interaction with acylating reagents, even being able to act as an acylation catalyst. The dissolution can be achieved by two routes, viz. (1) by solvent exchange, meaning the cellulose is first suspended in water and subsequently transferred into organic liquids with decreasing polarity and finally DMA/LiCl [25], and (2) after heating a suspension of cellulose in DMA, vacuum evaporating about 1/5 of the liquid (containing most of the water) and addition of the LiCl after which, during cooling to room temperature, a clear solution is obtained. The amount of LiCl is in the range of 5–15 per cent (w/w). The thermal cellulose activation under reduced pressure is far superior to the costly and time-consuming activation by solvent exchange.

In recent years, the cellulose/DMA/LiCl system has been studied intensively to develop efficient methods appropriate even for industrial application [26, 27]. The dissolution procedure and acetylation conditions allow

Table 16.2

Acetylation of different cellulose types in *N*,*N*-dimethyl acetamide (DMA)/LiCl with acetic anhydride (18 h at 60°C, adapted from [26, 27])

Starting materials				Molar ratio		DS
Cellulose from	M_w (g mol^{-1})	α-Cellulose content (%)	I_c (%)	AGU	Acetic anhydride	
Bagasse	116 000	89	67	1	1.5	1.0
Bagasse	116 000	89	67	1	3.0	2.1
Bagasse	116 000	89	67	1	4.5	2.9
Cotton	66 000	92	75	1	1.5	0.9
Sisal	105 000	86	77	1	1.5	1.0

Table 16.3

Preparation of aliphatic esters of cellulose in *N*,*N*-dimethyl acetamide (DMA)/LiCl

Reaction conditions						Reaction product		Reference
Acid chloride	Molar ratio		Base	Time (h)	Temperature (°C)	DS	Solubility[a]	
	AGU	Agent						
Hexanoyl	1	1.0	Py	0.5	60	0.89	DMSO, NMP, Py	[119]
Hexanoyl	1	2.0	Py	0.5	60	1.70	Acetone, MEK, $CHCl_3$, AcOH, THF, DMSO, NMP, Py	
Lauroyl	1	2.0	Py	0.5	60	1.83	Py	
Stearoyl	1	1.0	Py	1	105	0.79	Acetone, MEK, $CHCl_3$, AcOH, THF, DMSO, NMP, Py	
Hexanoyl	1	8.0	TEA	8	25	2.8	DMF	[26]
Heptanoyl	1	8.0	TEA	8	25	2.4	Toluene	
Octanoyl	1	8.0	TEA	8	25	2.2	Toluene	
Phenylacetyl	1	15.0	Py	3/1.5	80/120	1.90	CH_2Cl_2	[120]
4-Methoxy-phenyl-acetyl	1	15.0	Py	3/1.5	80/120	1.8	CH_2Cl_2	
4-Tolyl-acetyl	1	15.0	Py	3/1.5	80/120	1.8	CH_2Cl_2	

[a] DMSO, dimethyl sulfoxide; NMP, *N*-methyl-2-pyrrolidone; Py, pyridine; MEK, methyl ethyl ketone; AcOH, acetic acid; THF, tetrahydrofurane; DMF, *N*,*N*-dimethyl formamide.

excellent control of the DS in the range 1–3. The reaction at 110°C for 4 h without additional base or catalyst gives products with almost no degradation of the starting polymer. The distribution of substituents in the order C-6 > C-2 > C-3 is determined by ^{13}C NMR spectroscopy. In addition to microcrystalline cellulose, cotton, sisal, and bagasse-based cellulose may serve as starting materials (Table 16.2). The crystallinity of the starting polymer has little effect on the homogeneous acetylation.

The acylation of cellulose with acid chlorides in DMA/LiCl is most suitable for the homogeneous synthesis of readily soluble partially functionalized long-chain aliphatic esters and substituted acetic acid esters (Table 16.3). In contrast to the anhydrides, the fatty acid chlorides are soluble in the reaction mixture and soluble polysaccharide esters may be formed with a very high efficiency. Even in the case of stearoyl chloride, 79 per cent of the reagent is consumed for the esterification of cellulose.

Table 16.4

Influence of the amount tetra-*n*-butylammonium fluoride (TBAF) trihydrate on the efficiency of the acetylation of sisal cellulose with acetic anhydride in dimethyl sulfoxide (DMSO)/TBAF (adapted from [28])

Per cent TBAF in DMSO	Cellulose acetate	
	DS	Solubility[a]
11	0.30	Insoluble
8	0.96	DMSO, Py
7	1.07	DMSO, Py
6	1.29	DMSO, DMF, Py

[a] Py, pyridine; DMF, *N,N*-dimethyl formamide.

DMSO/TBAF is highly efficient as a reaction medium for the homogeneous esterification of cellulose by transesterification and after the *in situ* activation (see below) of complex carboxylic acids. The acylation using acid chlorides and anhydrides is limited because the solution contains a certain amount of water caused by the use of the commercially available TBAF trihydrate and the residual moisture in the air-dried polysaccharides. Nevertheless, this system has shown a remarkable capacity for the esterification of lignocellulosic materials, for example, sisal fibres, which contain about 14 per cent hemicellulose [28]. The DS values of cellulose acetate prepared from these fibres with acetic anhydride in mixtures of DMSO/TBAF were found to decrease with increasing TBAF concentration from 6 to 11 per cent (Table 16.4), due to the increased rate of hydrolysis both of the anhydride and the ester moieties.

Partial dehydration of DMSO/TBAF is possible by vacuum distillation and reactions in the ensuing solvent lead to products comparable to those obtained in reactions of cellulose dissolved in anhydrous DMA/LiCl. In addition to these basic studies, the conversion of cellulose in DMSO/TBAF with more complex carboxylic acids (*e.g.* furan-2-carboxylic acid) via *in situ* activation with *N,N′*-carbonyldiimidazole (CDI) was studied (see Section 16.2.2.1.3).

16.2.2.1 Homogeneous acylation with in situ activated carboxylic acids

The synthetic approach of *in situ* activation of carboxylic acids is based on the preliminary reaction of the carboxylic acid with a specific reagent to give an intermediate reactive derivative which can be prepared prior to the reaction with cellulose or converted directly in a one-pot process. This approach opens the way to a broad variety of new esters, because for numerous acids, for example unsaturated or hydrolytically unstable ones, reactive derivatives such as anhydrides or chlorides simply cannot be synthesized. The mild reaction conditions applied for the *in situ* activation prevent common side reactions like pericyclic reactions, hydrolysis, and oxidation. Moreover, due to their hydrophobic character, numerous anhydrides are not soluble in organic media used for cellulose modification, resulting in unsatisfactory yields and insoluble products. In addition, the conversion of an anhydride is combined with the loss of half of the acid during the reaction. Consequently, *in situ* activation is much more efficient.

16.2.2.1.1 Activation with sulphonic acid chlorides

One of the early attempts of *in situ* activation was the reaction of carboxylic acids with sulphonic acid chlorides that was adopted for the homogeneous modification of cellulose. The exclusion of the base simplified the reaction medium and the isolation procedure [29]. There is an ongoing discussion about the mechanism which initiates esterification of cellulose with the carboxylic acids in the presence of *p*-toluenesulphonic acid chloride (TosCl). The mixed anhydride of *p*-toluenesulphonic acid (TosOH) and the carboxylic acid is favoured [30]. However, from ^{1}H NMR experiments with acetic acid/TosCl, it was concluded that a mixture of acetic anhydride (2.21 ppm) and acetyl chloride (2.73 ppm) was responsible for the high reactivity of this system (Figs 16.2 and 16.3).

Cellulose esters having alkyl substituents in the range of C_{12} (laurylic acid) to C_{20} (eicosanoic acid), can be obtained with almost complete functionalization (DS 2.8–2.9) within 24 h at 50°C in the presence of sulphonic acid chlorides in DMA/LiCl, using pyridine as a base [31]. This is also a general method for the *in situ* activation

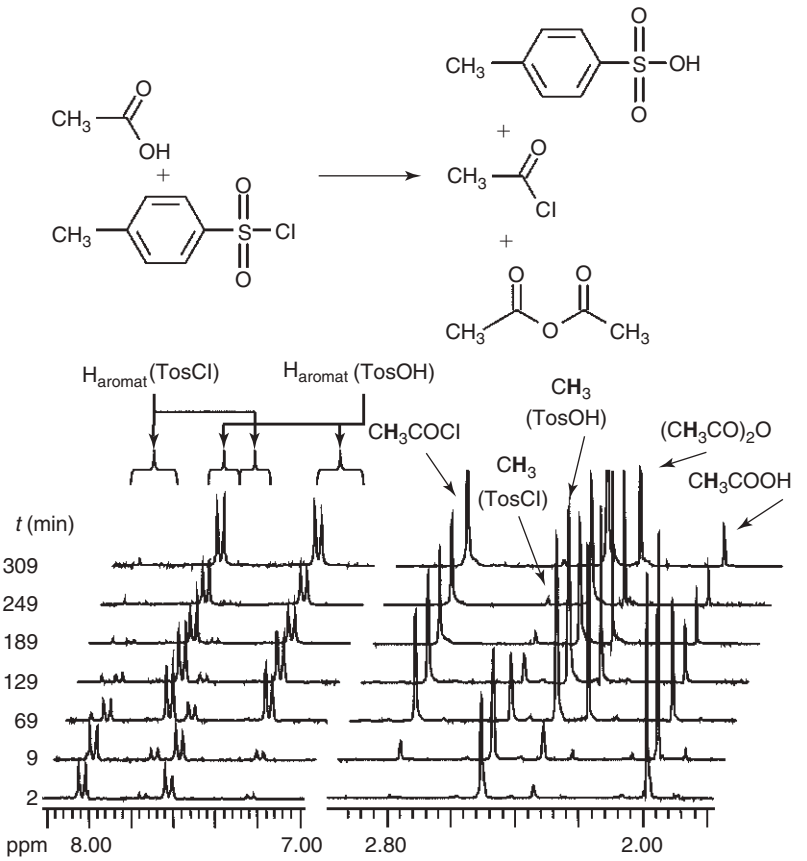


Figure 16.2 ^{1}H NMR spectroscopic investigation of the *in situ* activation of acetic acid with *p*-toluenesulphonic acid chloride showing the preferred formation of acetic anhydride and acetyl chloride (with permission from Springer, [1]).

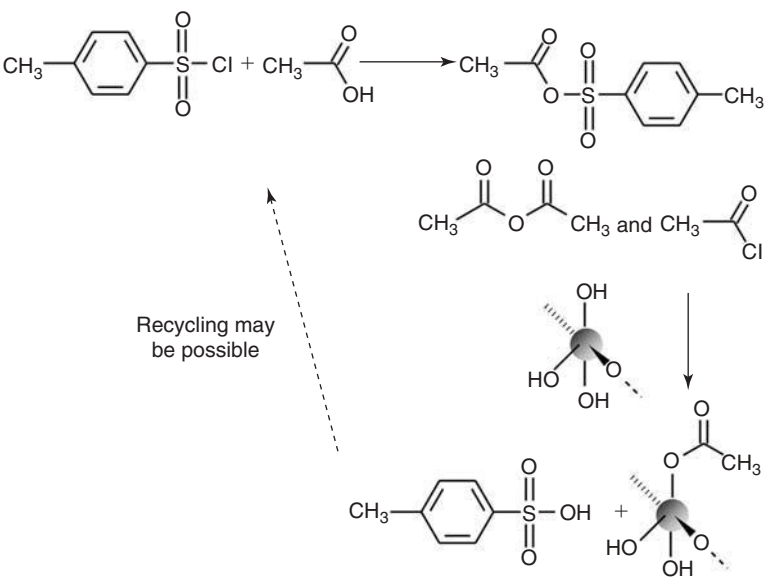


Figure 16.3 Schematic plot of the reaction by *in situ* activation of carboxylic acids with *p*-toluenesulphonic acid chloride (with permission from Springer, [1]).

Table 16.5

Esterification of cellulose dissolved in *N,N*-dimethyl acetamide (DMA)/LiCl mediated with TosCl with different carboxylic acids (adapted from [32])

Entry	Reaction conditions						Product	
	Carboxylic acid	Molar ratio					DS	Solubility in $CHCl_3$
		AGU[a]	Acid	TosCl[b]	Py[c]	Time (h)		
1	Lauric	1	2	2	0	24	1.55	+
2	Palmitic	1	2	2	0	24	1.60	+
3	Stearic	1	2	2	0	24	1.76	+
4	Lauric	1	2	2	4	24	1.79	+
5	Palmitic	1	2	2	4	24	1.71	+
6	Stearic	1	2	2	4	24	1.92	+
7	Lauric	1	2	2	0	4	1.55	+
8	Palmitic	1	2	2	0	4	1.50	+
9	Lauric	1	2	2	0	1	1.36	−
10	Palmitic	1	2	2	0	1	1.36	+

[a] Anhydroglucose unit.
[b] *p*-Toluenesulphonic acid chloride.
[c] Pyridine.

of waxy carboxylic acids. The reaction proceeds in 4 h to give partially functionalized fatty acid esters of a maximum DS (see entries 1, 7, and 9 in Table 16.5). The addition of an extra base further increases the DS by 0.1–0.2 units (see entries 1–3 and 4–6 in Table 16.5 [32]).

The *in situ* activation with TosCl is also useful for the introduction of fluorine-containing substituents, for example, 2,2-difluoroethoxy-, 2,2,2-trifluoroethoxy-, and 2,2,3,3,4,4,5,5-octafluoropentoxy functions with DS values mainly in the range of 1.0–1.5, leading to a stepwise increase in the lipophilicity of the products and an increase in their thermal stability. Structure analysis is possible by ^{19}F NMR spectroscopy [30, 33, 34]. Moreover, the *in situ* activation with TosCl enables the synthesis of water soluble cellulose esters by derivatization of cellulose with oxacarboxylic acids in DMA/LiCl [29]. The conversion of the polysaccharide with 3,6,9-trioxadecanoic acid or 3,6-dioxaheptanoic acid, in the presence of TosCl, yields non-ionic cellulose esters with DS values in the range of 0.4–3.0. In this case, the esterification is carried out without an additional base. The cellulose derivatives start to dissolve in water at a DS value as low as 0.4 and they are also soluble in common organic solvents such as acetone or ethanol.

16.2.2.1.2 Activation with dialkylcarbodiimide

Coupling reagents of the dialkylcarbodiimide type, in particular dicyclohexylcarbodiimide (DCC), are most frequently utilized for the esterification of polysaccharides with complex carboxylic acids. These reagents have a number of drawbacks: (1) they are toxic (the LD_{50} dermal, rat, of DCC is 71 mg/kg); (2) the *N,N′*-dialkylurea formed during the reaction is hard to remove from the polymer, except if it is formed in DMF and DMSO where it can be filtered off; (3) in the case of esterification in DMSO in the presence of these reagents, the oxidation of the hydroxyl functions, via the Moffatt type, may occur.

Another strategy for *in situ* activation, used for the synthesis of aliphatic cellulose esters, is the exploitation of DCC in combination with 4-pyrrolidinopyridine (PP) [35]. Among the advantages of this method are the high reactivity of the intermediately formed mixed anhydride with PP and a completely homogeneous reaction in DMA/LiCl up to hexanoic acid. If the reaction is carried out with the anhydrides of carboxylic acids, the carboxylic acid liberated is recycled by forming the mixed anhydride with PP, which is applied only in a catalytic amount. The toxic DCC can be recycled from the reaction mixture (Fig. 16.4). In addition, the method is utilized to obtain unsaturated esters (*e.g.* methacrylic-, cinnamic-, and vinyl acetic acid esters) and esters of aromatic

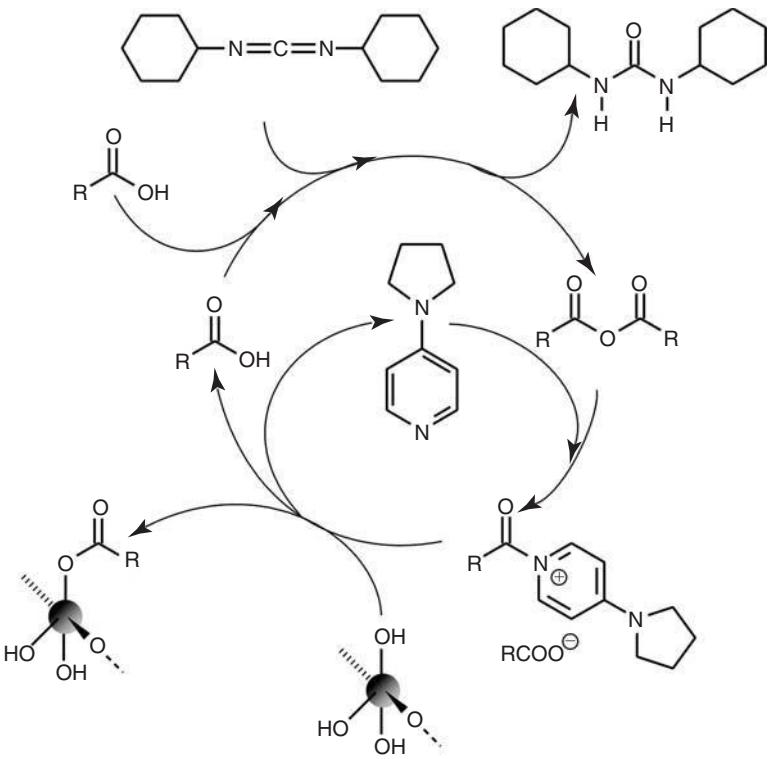


Figure 16.4 Scheme of the esterification of cellulose using PP/DCC (with permission from Springer, [1]).

carboxylic acids including cellulose (*p-N,N*-dimethylamino)benzoate. Here, the amino group containing ester can be converted to a quaternary ammonium derivative, which imparts water solubility to the material [36, 37].

16.2.2.1.3 Activation with *N,N′*-carbonyldiimidazole

A method with an enormous potential for polysaccharide modification is the homogeneous one-pot synthesis after *in situ* activation of the carboxylic acids with CDI, well known from organic chemistry [38]. It is especially suitable for the functionalization of the biopolymers because during the conversion the reactive imidazolide of the acid is generated and non-toxic by-products formed are CO_2 and imidazole only (Fig. 16.5). The imidazole is freely soluble in a broad variety of solvents including water, alcohol, ether, chloroform, and pyridine and can be easily removed. In addition, the pH is not drastically changed during the reaction resulting in negligible chain degradation.

The synthesis is generally carried out as a one-pot reaction in two steps. The acid is transformed with the CDI to give the imidazolide. The conversion of the alcohol in the first step is also possible for the esterification but yields undesired crosslinking by carbonate formation in case of a polyol like cellulose (see Fig. 16.5). Model reactions and NMR spectroscopy with acetic acid confirm that during a treatment at room temperature CDI is completely consumed within 6 h (see Fig. 16.5). Thereby, the tendency of crosslinking of remaining CDI leading to insoluble products is avoided. For instance, the reaction with (-)-menthyloxyacetic acid *in situ* activated with CDI can be carried out simply by mixing the solution of the imidazolide prepared in DMA and the cellulose in DMA/LiCl and increasing the temperature to 60°C. Pure (-)-menthyloxyacetic acid esters of cellulose with DS as high as 2.53 are obtained by precipitation in methanol and filtration (Table 16.6). The cellulose esters are characterized regarding structure and DS by means of FTIR spectroscopy, elemental analysis, ^{1}H- and ^{13}C NMR spectroscopy, and additionally by ^{1}H NMR spectroscopy after peracetylation [39]. A wide variety of carboxylic acids with, for example, unsaturated, 3-(2-furyl)-acrylcarboxylic acid, heterocyclic, furan-2-carboxylic acid, and crown ether, 4′-carboxybenzo-18-crown-6, are available by the conversion of cellulose via this path (Table 16.6).

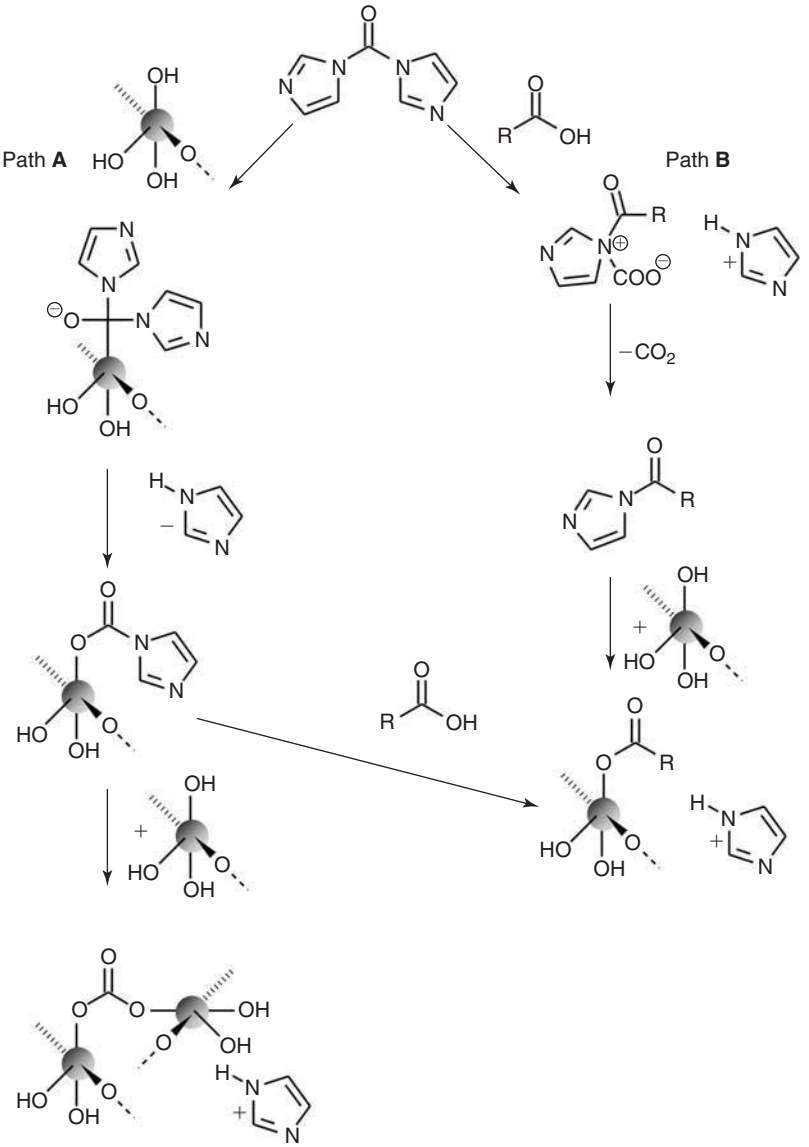


Figure 16.5 Reaction paths leading exclusively to esterification (path B) or crosslinking (path A) if the polysaccharide is treated with CDI in the first step (with permission from Springer, [1]).

In contrast, the conversion of cellulose with camphor-10-sulphonic acid via *in situ* activation with CDI is not efficient to obtain a chiral sulphonic acid ester of cellulose. Only very small amount of sulphonic acid ester functions can be introduced in agreement with results of the chemistry of low molecular weight alcohols regarding a much lower efficiency of CDI for the preparation of sulphonic acid esters [38].

In addition to DMA/LiCl, DMSO/TBAF is an appropriate reaction medium for homogeneous acylation of cellulose applying *in situ* activation with CDI. Results of reactions of cellulose with acetic-, stearic-, adamantane-1-carboxylic-, and furan-2-carboxylic acid imidazolides are summarized in Table 16.7.

16.2.2.1.4 Activation with iminium chlorides

A mild and efficient method is the *in situ* activation of carboxylic acids via iminium chlorides that are simply formed by reaction of DMF with a variety of chlorinating agents including phosphoryl chloride, phosphorus

Table 16.6

Esterification of cellulose with (-)-menthyloxyacetic acid, furan-2-carboxylic acid, 3-(2-furyl)-acrylcarboxylic acid, and 4′-carboxybenzo-18-crown-6 via *in situ* activation with *N*,*N*′-carbonyldiimidazole (CDI) (adapted from [39])

Conditions					Product		
Entry	Carboxylic acid	Molar ratio			DS	Solubility[b]	
		AGU[a]	Acid	CDI		DMSO	DMF
1	(-)-Menthyloxyacetic	1	2.5	2.5	0.20	+	+
2		1	5.0	5.0	1.66	−	+
3		1	7.5	7.5	2.53	−	+
4	3-(2-Furyl)-acryl-	1	2.5	2.5	0.52	+	−
5		1	5.0	5.0	1.14	+	+
6		1	7.5	7.5	1.52	+	−
7	Furan-2-	1	2.5	2.5	0.80	+	+
8		1	5.0	5.0	1.49	+	+
9		1	7.5	7.5	1.97	+	+
10	(Benzo-18-crown-6)-4′-	1	2.3	2.3	0.40	+	−

[a] Anhydroglucose unit.
[b] DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethyl formamide.

Table 16.7

Homogeneous acylation of cellulose dissolved in dimethyl sulfoxide (DMSO)/tetra-*n*-butylammonium fluoride (TBAF) with different carboxylic acids, mediated by *N*,*N*′-carbonyldiimidazole (CDI)

Entry	Conditions				Product	
	Carboxylic acid	Molar ratio			DS	Solubility[b]
		AGU[a]	Acid	CDI		
1	Acetic	1	3	3	0.51	DMSO, DMA
2	Stearic	1	2	2	0.47	DMSO
3	Stearic	1	3	3	1.35	DMSO
4	Adamantane-1-carboxylic	1	2	2	0.50	DMA/LiCl
5	Adamantane-1-carboxylic	1	3	3	0.68	DMSO, DMA
6	Furan-2-carboxylic	1	3	3	1.91	DMSO, DMA, Py

[a] Anhydroglucose unit.
[b] DMA, *N*,*N*-dimethylacetamide; Py, pyridine.

trichloride and, most frequently, oxalyl chloride and subsequent reaction with the acid. During the reaction of acid iminium chlorides with alcohols mostly gaseous side products are formed and the solvent is regenerated (Fig. 16.6, Table 16.8 [40]). In a 'one-pot reaction', acylation of cellulose with the long-chain aliphatic acids (stearic acid and palmitic acid), the aromatic acid 4-nitrobenzoic acid and adamantane-1-carboxylic acid are easily achieved. NMR spectroscopy reveals that the conversion succeeds with quantitative yield in case of acetic acid.

16.2.3 Regioselectively functionalized cellulose esters

Conversion of 6-*O*-trityl cellulose (see Chapter 4) with acetic acid anhydride or propionic acid anhydride and subsequent detritylation by treating the completely functionalized polymers with HBr in acetic acid yields the

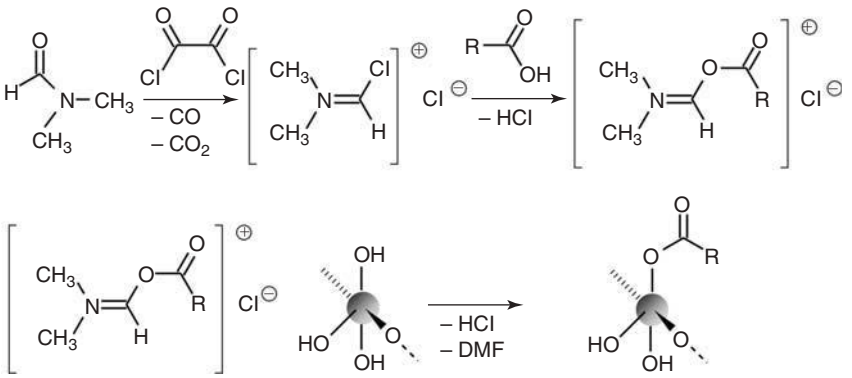


Figure 16.6 Preparation of cellulose esters via *in situ* activation by iminium chlorides (with permission from Springer, [1]).

Table 16.8

Esterification of cellulose dissolved in *N*,*N*-dimethyl acetamide (DMA)/LiCl via the iminium chlorides of different carboxylic acids (adapted from [40])

Entry	Carboxylic acid	Molar ratio			Product	
		AGU[a]	Acid	Oxalyl chloride	DS	Solubility[b]
1	Stearic	1	3	3	0.63	DMSO/LiCl
2	Stearic	1	5	5	1.84	THF, $CHCl_3$
3	Palmitic	1	6	6	1.89	DMSO, DMA, THF
4	Adamantane-1-carboxylic	1	1	1	0.47	DMSO/LiCl
5	Adamantane-1-carboxylic	1	3	3	1.20	DMA, DMSO, DMF
6	4-Nitrobenzoic	1	1	1	0.30	DMSO/LiCl
7	4-Nitrobenzoic	1	2	2	0.52	DMSO
8	4-Nitrobenzoic	1	3	3	0.94	DMSO

[a] Anhydroglucose unit.

[b] DMSO, dimethyl sulfoxide; THF, tetrahydrofurane; DMF, *N*,*N*-dimethyl formamide.

corresponding 2,3-*O*-functionalized esters [41]. Subsequent acylation of the hydroxyl groups at position 6 leads to the peracylated cellulose esters with inverse functionalization pattern useful for the peak assignment of NMR spectra. The cellulose esters form single crystals visualized by means of AFM [42]. Investigations of the structure in solution revealed large differences to cellulose esters with random distribution of substituents, for example, the chain conformation, solubility, and clustering mechanism is different [43, 44].

16.3 NUCLEOPHILIC DISPLACEMENT REACTIONS WITH CELLULOSE

It is well known that hydroxyl functions are converted to a good leaving group by the formation of the corresponding sulphonic acid esters and hence nucleophilic displacement reactions are possible. Advantageously, the formation of sulphonic acid esters is carried out homogeneously by conversion with sulphonic acid chlorides in the presence of a tertiary organic base in DMA/LiCl. In particular, homogeneous tosylation applying TosCl in the presence of triethylamine is very efficient [25, 45, 46].

Table 16.9

Selected applications of deoxycellulose (adapted from [52] and references herein, see details there)

Application field	Functionalization
Biological	
Bacteriostatic	Different cellulosics including deoxycellulose treated with 5-nitrofuroyl chloride
Immobilization of enzymes	Aminodeoxy cellulose
Anticoagulants	Sulfated aminodeox cellulose
Enzyme purification	Aminodeoxy cellulose
Chemical	
Removal of heavy metals, for example,	
Hg	Chlorodeoxy cellulose + ethylenediamine, thiourea, thiosemicarbazide, thioacetamide
Cu, Mn, Co, Ni	6-Chlorodeoxy cellulose + diamines
Hg, Ag	S-substituted 6-deoxy-6-mercapto cellulose
Preconcentration of trace elements	Tosyl cellulose treated with piperazine and CS_2 (cellulose piperazinedithiocarboxylate)
Sorption of uranium	Cellulose piperazinedithiocarboxylate
Chelating and complexing agents	Chlorodeoxy cellulose + iminodiacetic acid
	Schiff base formation from aminodeoxy cellulose
Physical	
Flame retardants	Chlorodeoxy cellulose
Propellants	Azidodeoxy cellulose
Electrostatic printing	Different cellulosics including aminodeoxy derivatives
Electron transfer catalysts	Viologens from ethyl cellulose derivatives

Applying S_N reactions, deoxycellulose products are available where the hydroxyl groups of the AGU are partially or completely replaced by other functional groups, that is, the reaction takes place at the carbon atoms. Considerable interest has found halodeoxycelluloses as starting material for S_N reactions yielding cellulose derivatives with interesting properties (Table 16.9) [47–52]. Recent studies comprise the introduction of amine/ammonium functions either by introduction of azide moieties and subsequent reduction or by using various di- and tri-amines for the S_N reaction.

The S_N reaction of tosyl cellulose with NaN_3 was comprehensively studied. It appears that not only the primary but also the secondary tosylates may be substituted. Consequently, the DS of azide moieties exceeds a value of 1, which is not possible with most of the nucleophils studied [53]. The reduction of the azide moiety is possible either with $LiAlH_4$ [54] or $NaBH_4/CoBr_2$/2,2′-bipyridine [55].

Moreover, a number of aminodeoxycellulose were accessible. Water soluble 6-trialkylammonium-6-deoxycellulose could be prepared [56]. Conversion of cellulose tosylate with diamines like 1,4-phenylenediamine yields polymers that can be applied for the immobilization of enzymes by diazo coupling, for example [57].

16.4 ETHERIFICATION OF CELLULOSE

Etherification is a very important branch of commercial cellulose derivatization. Cellulose ethers are prepared in technical scale by conversion of alkali cellulose with alkylating reagents, for example epoxides, alkyl- and carboxymethyl halides [58, 59].

In the case of heterogeneous reactions, the reactivity and the accessibility of the hydroxyl groups are determined by hydrogen bond-breaking activation steps and by interaction with the reaction media [59, 60]. Additionally, the reaction of cellulose with reagents of low steric demand leads to a random distribution of substituents within the AGU and along the polymer chain. It is well known that not only the DS but also the substitution pattern may influence the properties of cellulose ethers [61]. However, to gain detailed information about the influence of the structures on properties not only a comprehensive structure characterization but also cellulose ethers with a defined distribution of the functional groups are indispensable for the establishment of the structure–property relationships.

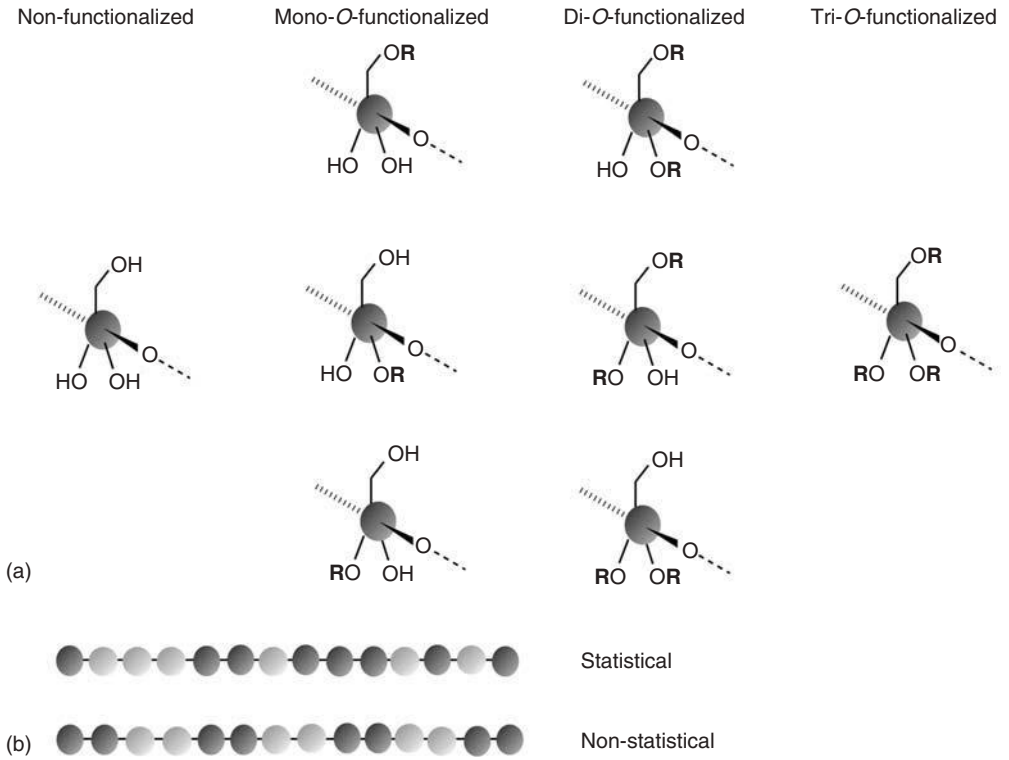


Figure 16.7 Distribution of the functional groups by regiocontrolled synthesis of cellulose derivatives: (a) within the anhydroglucose units and (b) along the polymer chain.

16.4.1 Regioselectively functionalized cellulose ether

'Regioselectivity' in cellulose chemistry means an exclusive or significant preferential reaction at one or two of the three positions 2, 3, and 6 of the AGU as well as along the polymer chain (Fig. 16.7).

In general, by chemical modification reactions of cellulose no equal reactivity of positions 2, 3, and 6 appears. For instance, homogeneous acylation in DMA/LiCl results in products with a preferred functionalization at position 6, while the carboxymethylation in 2-propanol/aqueous NaOH proceeds faster at position 2 compared to 6 and 3.

An important area in cellulose chemistry is the definite control of the substitution within the repeating unit. Up to now, the most important approach in this regard is the application of protecting groups (Fig. 16.8(a)). Other methods comprising, for example, selective cleavage of primary substituents by chemical or enzymatic treatment [20, 62, 63] play a minor role. An example is the deacetylation of cellulose acetate under aqueous acidic or alkaline conditions or in the presence of amines (Fig. 16.8(b)). In addition, activating groups like the tosyl moiety may also be disposed for selective reactions (see Section 16.3).

The most widely used protecting group is the triphenylmethyl (trityl) moiety (Fig. 16.9). Heterogeneous introduction of the trityl groups starts with an activated polymer obtained either by the deacetylation of cellulose acetate [64, 65] or by mercerization of cellulose [66] followed by a conversion in anhydrous pyridine. Furthermore, tritylation of cellulose yielding polymers with DS values of 1.0 proceeds in DMA/LiCl and DMSO/SO_2/diethylamine (DEA) [67, 68]. Methoxysubstituted triphenylmethyl compounds are more effective protecting groups for the primary hydroxyl group of cellulose [69]. The reaction of cellulose dissolved in DMA/LiCl with 4-monomethoxytriphenylmethyl chloride is 10 times faster than the conversion with unsubstituted trityl chloride. Complete functionalization of the primary hydroxyl groups is possible within 4 h and 70°C. Even after long reaction times, excess of the reagent, and elevated temperatures, alkylation of the positions 2 and 3 was less than 11 per cent, which is the same range as for the unsubstituted trityl function. Moreover, the detritylation occurs 20 times faster [61].

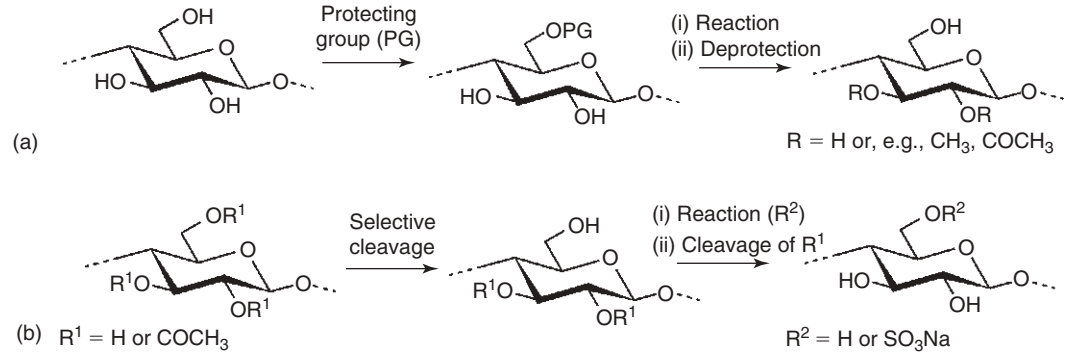


Figure 16.8 Pathways for the regioselective functionalization: (a) protecting group technique, (b) selective cleavage of primary substituents.

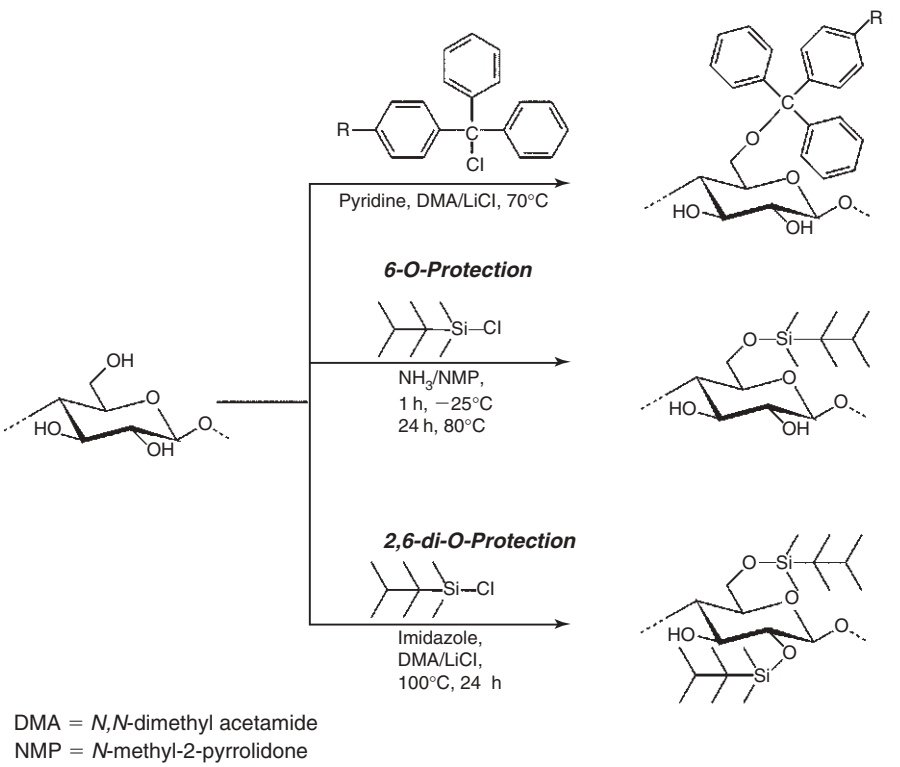


Figure 16.9 Protecting group technique: tritylation with trityl chloride in DMA/LiCl or silylation with thexyldimethylchlorosilane in NMP/ammonia for the regioselective blocking of the primary OH group and silylation in DMA/LiCl to protect the 6 and 2 positions simultaneously.

Trialkyl- (with at least one bulky alkyl moiety) and triaryl-silyl groups were investigated to protect the primary groups of cellulose. Pawlowski synthesized tert-butyldimethylsilyl cellulose with a DS of 0.68 in DMA/LiCl with mostly functionalization of position 6 [70]. Among this type of derivatives, 6-*O*-thexyldimethylsilyl (TDS) cellulose is most suitable (Fig. 16.9), [71, 72]. A synthesis starting with a heterogeneous phase reaction in ammonia-saturated polar aprotic liquids at −15°C by conversion of the cellulose with TDS chloride leads to a specific state of dispersion after evaporation of the ammonia at about 40°C, which does not permit any further reaction of the secondary hydroxyl groups, even with a large reagent excess, increased temperature, or long reaction time [71].

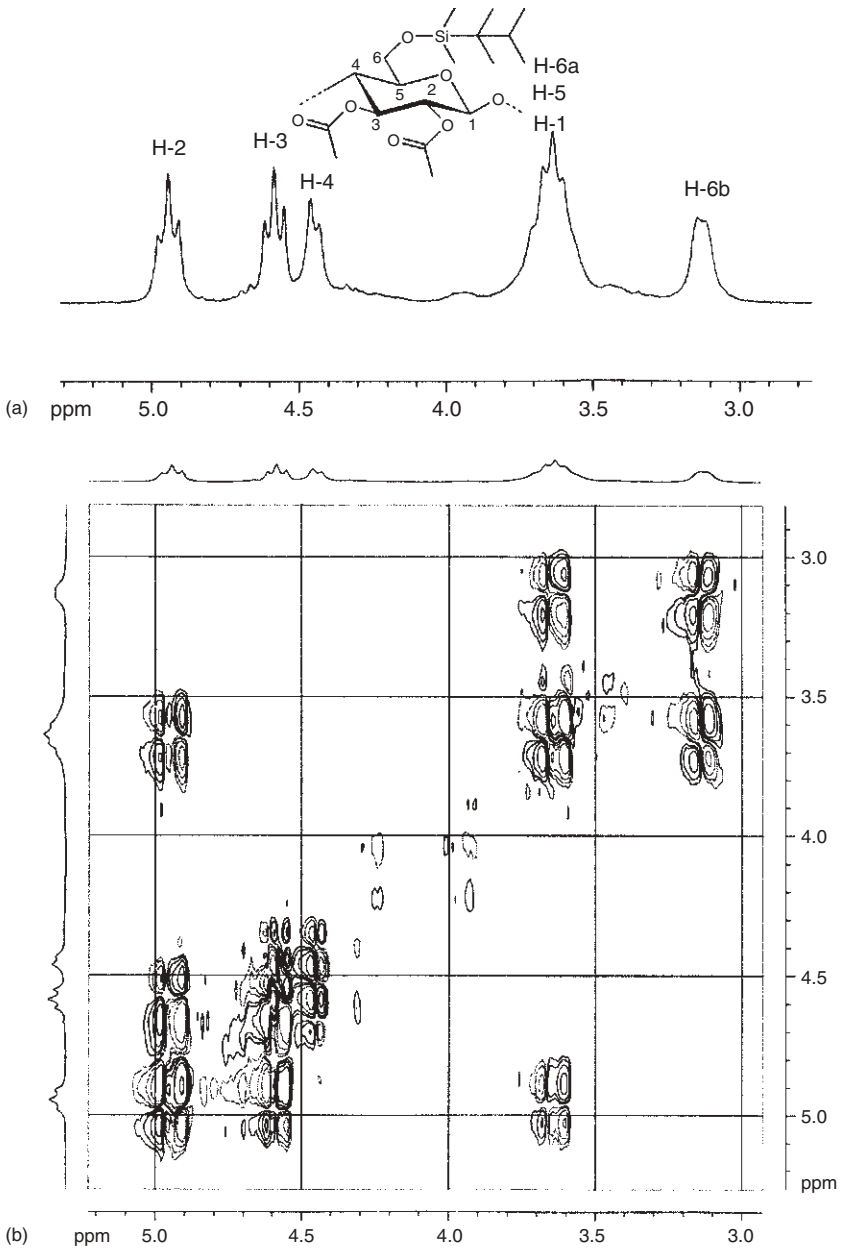


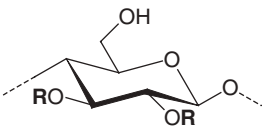
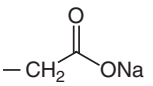
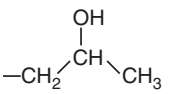
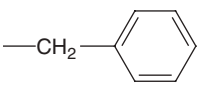
Figure 16.10 ^{1}H NMR spectrum (a) and $^{1}H/^{1}H$ correlated NMR spectrum (b) of peracetylated TDS cellulose in $CDCl_3$: assigned cross-peaks of the anhydroglucose unit [2,3-*O*-Ac-6-*O*-TDS] (Ac = acetyl, with permission from Springer, [71]).

The degree of TDS groups introduced by homogeneous silylation in DMA/LiCl to a total value of 0.99 was determined to be 85 per cent at position 6 only (GC/MS analysis). However, the homogeneous reaction in DMA/LiCl can be used to synthesize a 2,6-di-*O*-TDS cellulose [61, 72].

The structural uniformity and regioselectivity of the silylated cellulose products were demonstrated by means of one- and two-dimensional NMR techniques (Fig. 16.10) after subsequent acetylation of the remaining hydroxyl groups [68, 71] or after permethylation of the residual OH groups and chain degradation by means of HPLC and GC-MS [66, 69, 72, 73].

Table 16.10

Examples for regioselectively functionalized 2,3-di-*O*-cellulose ethers

	R	DS	Via[a]	Reference
2,3-Di-*O*-methyl cellulose	—CH_3	Up to 1.77	Trt	[65]
		2.0	Trt	[66, 83]
		2.0	TDS	[72]
2,3-Di-*O*-ethyl cellulose	—CH_2—CH_3	Up to 1.93	Trt	[65]
2,3-Di-*O*-CM cellulose		0.054–1.07	Trt	[75]
		Up to 1.91	MMTr	[74]
2,3-Di-*O*-hydroxyethyl cellulose		Up to MS 2.0	MMTrt	[76b]
2,3-Di-*O*-hydroxypropyl cellulose		Up to MS 2.0	MMTrt	[76b]
2,3-Di-*O*-allyl cellulose	—$CH_2CH{=}CH_2$	2.0	Trt	[78, 82]
2,3-Di-*O*-benzyl cellulose		2.0	Trt	[78, 82]
2,3-Di-*O*-octadecyl cellulose	—$(CH_2)_{17}CH_3$	2.0	Trt	[84]

[a] Trt, trityl; TDS, thexyldimethylsilyl; MMTrt, 4-monomethoxytrityl.

16.4.1.1 2,3-Di-O-ethers of cellulose

The 6-mono-*O*-trityl cellulose or the more efficient 6-*O*-mono-*O*-(4-monomethyoxytrityl) derivative, and 6-mono-*O*-TDS cellulose were used to synthesize regioselectively functionalized cellulose ethers at positions 2 and 3 after the exclusive cleavage of the protecting groups (Table 16.10). In case of the trityl derivatives, the deprotection is carried out most efficiently with HCl in a suitable solvent. For TDS protected derivatives, tetrabutylammonium fluoride in THF is most successful for the cleavage of the silyl groups.

The alkylation of the 6-*O*-trityl cellulose was achieved in DMSO with solid NaOH as base and the corresponding alkyl halides at 70°C within several hours. Interestingly, a small amount of water in the mixture (about 1 ml per 60 ml DMSO) increases the conversion up to a nearly complete functionalization of the secondary hydroxyl groups [65]. The synthesis of ionic 2,3-*O*-carboxymethyl cellulose (CMC) was carried out with sodium monochloroacetate as etherifying reagent in the presence of solid NaOH in DMSO and detritylation with gaseous HCl in dichloromethane for 45 min at 0°C or alternatively with aqueous hydrochloric acid in an ethanol slurry [74]. The 2,3-*O*-CMC synthesized possess DS values up to 1.91, which shows water solubility at a DS of 0.3 [75]. 2,3-Di-*O*-hydroxyethyl- (HEC) and 2,3-di-*O*-hydroxypropyl celluloses (HPC) were synthesized by heterogeneous etherification of 6-*O*-(4-monomethyoxytrityl) cellulose (MMTC) with alkylene oxide in a 2-propanol/10 per cent NaOH–water mixture. Due to the very hydrophobic character of MMTC, the reaction was successful in the presence of anionic and non-ionic detergent in the reaction mixtures [76]. The polymers become water soluble starting with a molecular degree of substitution (MS) 0.25 (HEC) and 0.50 (HPC), while a conventional HPC is water soluble with MS > 4. ^{13}C NMR spectroscopy revealed the etherification of the secondary hydroxyl groups of the AGU. Only one signal can be observed for the CH_2 group of position 6 (Fig. 16.11). In addition, the peaks of the etherified positions 2 and 3 appear in the range of 80–83 ppm.

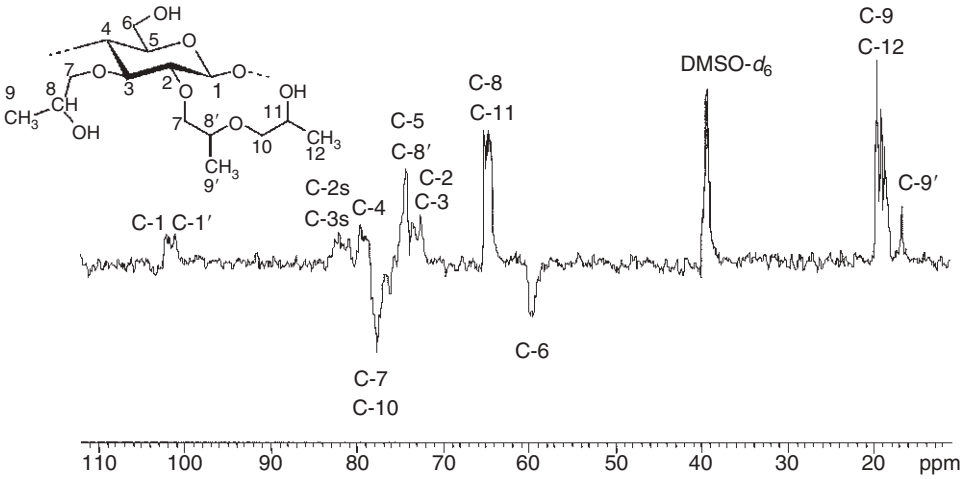


Figure 16.11 ^{13}C DEPT 135 NMR spectrum of 2,3-*O*-hydroxypropyl cellulose (molar substitution 0.33) recorded in DMSO-d_6 at 40°C (with permission from Wiley-VCH, [76b]).

Cellulose ethers may exhibit the phenomenon of thermoreversible gelation that strongly depends on the functionalization pattern as demonstrated for selectively methylated cellulose [77]. A series of 2,3-*O*-methyl celluloses was prepared starting from 6-*O*-trityl- and 6-*O*-monomethoxytrityl cellulose [66]. The total DS and the composition of the repeating units are slightly different according to varied reaction conditions. The thermal events are in strong correlation with the polymer composition concerning the different functionalization pattern of the repeating units. In case of a polymer containing tri-*O*-methylated glucose units in combination with monomethylated ones, a distinct thermal behaviour was found, that is, the methyl cellulose shows thermoreversible gelation. In contrast, a uniform 2,3-*O*-methyl cellulose shows no thermal gelation. It becomes obvious that the 2,3-*O*-methyl glucose units do not affect significantly intermolecular interactions that are necessary for the gelation.

The methylation of the 6-*O*-TDS cellulose with methyl iodide and NaH in THF yields a water insoluble product that dissolves only in NMP and methanol/chloroform in contrast to a 2,3-*O*-methyl cellulose prepared via 6-*O*-trityl cellulose [72]. Thus, the structure is more uniform compared with samples prepared via tritylation.

16.4.1.2 6-O-ethers of cellulose

Up to now, the only path to 6-*O*-cellulose ethers is the time-consuming synthesis described by Kondo [78], which comprises two different protecting groups: 6-*O*-trityl cellulose is converted with allyl chloride in the presence of NaOH resulting in a complete functionalization of positions 2 and 3; subsequent detritylation and isomerization of the allyl groups to 2,3-*O*-(1-propenyl) substituents with potassium tert-butoxide, alkylation of position 6 followed by the cleavage of the 1-propenyl groups at positions 2 and 3 with HCl in methanol. The 6-*O*-alkyl cellulose samples were included in investigations about gelation [79], about blends with synthetic polymers [80, 81], about hydrogen bond system [82, 83], about the behaviour of Langmuir–Blottgett monolayers [84], and about enzymatic degradation [85].

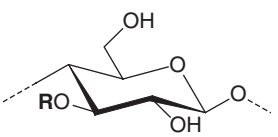
16.4.1.3 3-O-ethers of cellulose

The conversion of 2,6-di-*O*-TDS cellulose with an excess of alkyl halides in THF in the presence of NaH afforded the fully etherified polymers that can be desilylated with fluoride ions yielding 3-mono-*O*-functionalized cellulose ethers (Table 16.11) [86–89].

3-Mono-*O*-methyl cellulose swells in polar media like DMSO indicating a strong network of hydrogen bonds. It becomes soluble by addition of LiCl that destroys intermolecular interactions. An increase of the length of the alkyl chains changes the solubility of the 3-mono-*O*-alkyl celluloses from water (C_2) via aprotic dipolar solvents (up to C_5 alkyl chains) to non-polar solvents like THF for C_5–C_{12} alkyl chains. Light scattering investigations of 3-*O*-*n*-pentyl-, 3-*O*-*iso*-pentyl-, and 3-*O*-dodecyl cellulose in THF disclosed a different aggregation behaviour.

Table 16.11

3-*O*-mono-alkyl celluloses and their solubility

 R	Solubility[a]					Reference
	Ethanol	DMSO	DMA	THF	H_2O	
—CH_3	−	−	−	−	−	[86]
—CH_2—CH_3	−	+	+	−	+	[88]
—CH_2—CH═CH_2	−	+	+	−	−	[86]
—CH_2—CH_2—O—CH_3	−	+	+	−	+	[89]
—CH_2—CH_2—CH_3	+	+	+	−	−	[89]
—CH_2—CH_2—CH_2—CH_3	−	+	+	−	−	[89]
—CH_2—CH_2—CH_2—CH_2—CH_3	+	+	+	+	−	[87]
—CH_2—CH_2—CH—$(CH_3)_2$	+	+	+	+	−	[87]
—$(CH_2)_{11}$—CH_3	−	−	−	+	−	[87]

[a] *N*,*N*-dimethyl acetamide (DMA), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), soluble (+), insoluble (−).

Whereas the 3-*O*-dodecyl cellulose forms molecularly dispersed solutions (at concentration less than 2 mg/l), the C_5 ethers show aggregation numbers of 6.5 (*iso*-pentyl) and 83 (*n*-pentyl) [87].

Ethyl cellulose of different functionalization pattern shows interesting thermoreversible gelation. While ethyl cellulose with DS 0.7–1.7 becomes water insoluble already at about 30°C [90], ethyl cellulose, which was prepared via induced phase separation (conversion of cellulose dissolved in DMA/LiCl with solid NaOH and ethyl iodide), possesses a distinct higher cloud point temperature of 56°C. A block-like distribution of substituents along the polymer chain is assumed for this polymer as described for CMC and MC [91, 92]. A similar value (63°C) was determined for the structural uniform 3-mono-*O*-ethyl cellulose independent of the DP [89].

One- and two-dimensional NMR spectroscopy demonstrates the uniform structure of the 3-*O*-alkyl ethers after peracetylation of the remaining OH groups as shown in Fig. 16.12 for 3-*O*-methoxyethyl-2,6-di-*O*-acetyl cellulose as a typical example [89].

16.4.2 Cellulose ethers with non-statistical distribution of functional groups

Different routes for the synthesis of cellulose ethers with a non-statistical distribution of the ether groups along the polymer chain are described. The concept of induced phase separation proceeds with the activation of cellulose by dissolution (*e.g.* in DMA/LiCl) and subsequent addition of solid water-free NaOH particles [92]. This process leads to reactive microstructures due to the regeneration of cellulose II on the interface solid particle/solution, which is shown by means of FTIR and polarizing light microscopy [91]. In this state, the reaction of cellulose with sodium monochloroacetate yields CMC with DS values up to 2.2 in a one-step procedure. The analysis of the CMC products can be realized by an HPLC separation of the repeating units after a complete depolymerization of the polymer backbone (preferably with $HClO_4$) [93] and a comparison of the mol fractions measured with values calculated by a simple statistics first described by [94], which simulated the conversion along the polymer chain without preference of any OH groups and without the influence of the DS. While the statistic data meet the mol fractions of conventionally obtained CMC completely, the mol fractions of CMCs synthesized via phase separation show significant differences from the statistical prediction. The concept of the induced phase separation can be extended to other solvent systems like DMSO/TBAF [7, 95, 96] and NMNO [4], various cellulose intermediates, for example, CA and TMSC as well as other cellulose ether syntheses [2] (Fig. 16.13).

CMC with a non-statistic functionalization pattern may be obtained by the concept of reactive structure fractions, which deals with the selective reaction in the activated non-crystalline areas of cellulose by treatment with low-concentrated aqueous NaOH [97, 98].

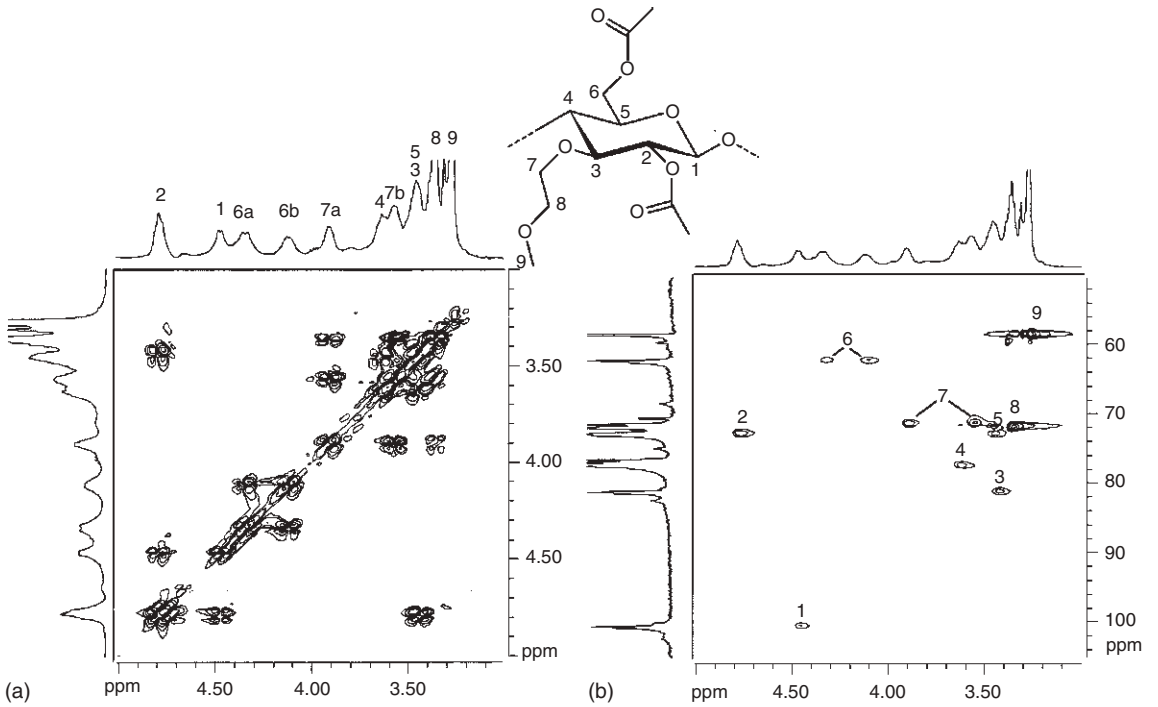


Figure 16.12 COSY (a) and HMQC NMR spectra (b) of peracetylated 3-*O*-methoxyethyl cellulose ($CDCl_3$) [89].

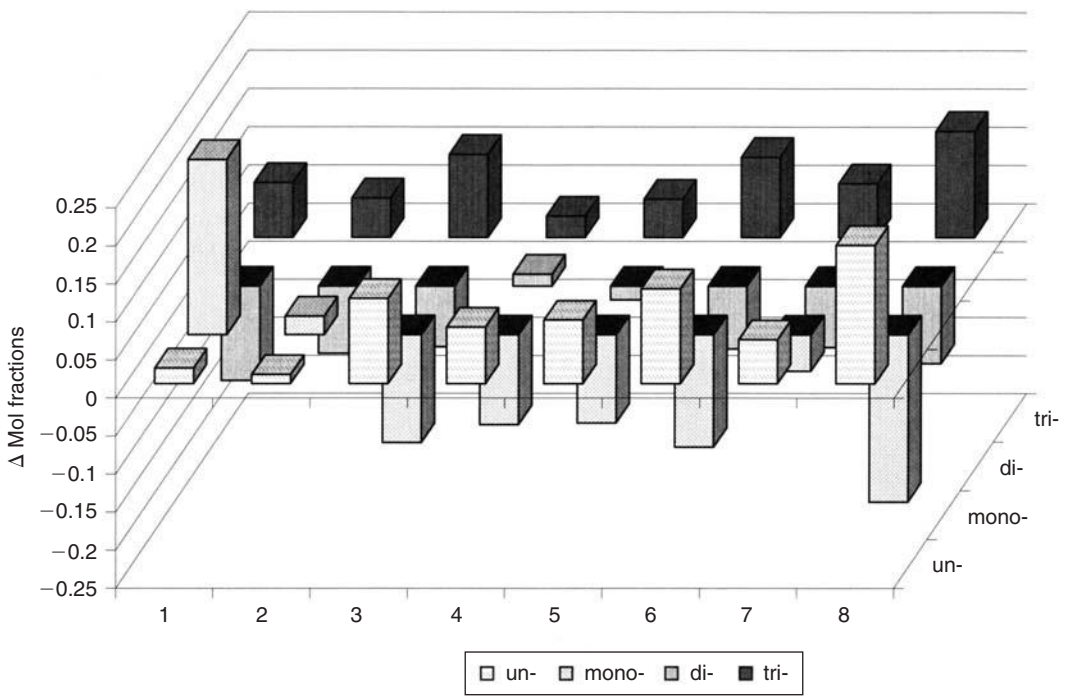


Figure 16.13 Absolute deviation of the mol fractions of un-, mono-*O*-, di-*O*-, and 2,3,6-tri-*O*-functionalized glucose from the binomial distribution of CMC and methyl cellulose (MC) synthesized via induced phase separation: **1** CMC synthesized in DMA/LiCl (DS 2.07); **2** CMC synthesized in DMSO/TBAF (DS 1.89), **3** CMC synthesized in *N*-methylmorpholine-*N*-oxide (DS 1.26), **4** CMC synthesized from cellulose acetate, **5** CMC synthesized from trimethylsilyl cellulose, **6** CMC synthesized from cellulose triflouroacetate, **7** synthesized from cellulose formiate, and **8** MC.

16.4.3 Completely functionalized cellulose ethers

Attempts to synthesize completely functionalized cellulose ethers applying both heterogeneous and homogeneous paths are known. A homogeneous synthesis path is the conversion of cellulose with alkyl halides in the presence of NaOH in the solvent system SO_2/DEA/DMSO [99]. Cellulose could be dissolved in DMSO/LiCl and alkylation is carried out in the presence of sodium dimsyl, resulting from the reaction of NaH and DMSO, with alkyl halides [100]. A facile method for the preparation of tri-*O*-alkyl cellulose deals with the conversion of cellulose acetate in DMSO using the alkyl halide in the presence of NaOH [101]. The complete methylation was described applying homogeneous etherification of cellulose in DMI/LiCl [102]. An interesting procedure for preparation of tri-*O*-allyl- and tri-*O*-crotyl cellulose uses solutions of cellulose in DMA/LiCl, the corresponding alkyl chlorides, and powdered NaOH [103]. The products were investigated regarding their thermal and mechanical properties [99, 103] and their structure in solution [87]. The tri-*O*-alkyl celluloses were characterized by means of different NMR methods involving DQF COSY, HSQC, TOCSY, and HMBC [103].

Fully functionalized trimethylsilyl cellulose (TMSC) was obtained with hexamethyldisilazane (HMDS) in liquid ammonia in a stainless steel autoclave at elevated temperature [104]. The completely silylated polymer exhibits unusual properties; it shows partial insolubility in THF, toluene, DMA, or cyclohexane compared to TMCS with a DS of 2.9 as a result of aggregation of the homopolymer structure. No glass transition was observed by DSC.

Highly functionalized TMSC was exploited for the continuous polymer fractionation [105]. Because of their stiff backbone with flexible side chains (so-called hair rod molecules) ultrathin films by Langmuir–Blodgett (LB) technique could be formed [106] yielding cellulose films after regeneration with HCl gas [107, 108].

16.4.4 Complete homogeneous etherification of cellulose

The first example for a totally homogeneous carboxymethylation is the conversion of cellulose in $Ni(tren)(OH)_2$, surprisingly leading to a CMC with a statistic functionalization pattern [4, 109]. Molten salts hydrates like $LiClO_4 \times H_2O$ were studied for homogeneous cellulose carboxymethylation [110]. Both synthesis paths result in CMCs with the same distribution of functions and properties, for example solubility, as products obtained in the slurry reaction. No complete functionalization was possible. A totally homogeneous hydroxyethylation is carried out using the solvent 6 wt per cent NaOH/4 wt per cent urea aqueous solution [111], which yields products with the analogous functionalization pattern as HEC obtained by the heterogeneous slurry process. An alternative path for cellulose etherification is the use of ionic liquids as reaction medium [22].

16.5 CONCLUSIONS

It appears that the application of dissolved cellulose opens new avenues for the design of cellulose-based products. Limitations of the application of solvents may result from high toxicity, high reactivity of the solvents leading to undesired side reactions, and the loss of solubility during reactions yielding inhomogeneous mixtures by formation of gels and pastes, which can be hardly mixed, and even by formation of de-swollen particles of low reactivity.

The combination with new solvents and esterification methodologies has driven the new synthetic paths for carboxylic ester formation of cellulose. The examples discussed illustrate the enormous structural diversity accessible by these new and efficient methods. Very promising solvents for homogeneous path chemistry of cellulose are ionic liquids even in a commercial scale that is intensively studied world-wide at present. In any case, the recycling of the solvents has to be addressed. In the field of cellulose ethers the regioselective introduction of the functional groups is still a synthetic challenge. These products will give new insights in the interaction of cellulose derivatives with each other, with other polymers and surfaces, and hence to improve common applications and to introduce cellulose-based materials in new application fields. Last but not least, it appears cellulose chemistry applying novel organic chemistry will lead to a variety of sophisticated products; impressive examples are S_N reactions. Moreover, Click chemistry with cellulose was already successfully realized [112].

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REFERENCES

1. Heinze T., Liebert T., Koschella A., *Esterification of Polysaccharides*, Springer Verlag, Berlin, Heidelberg, 2006.
2. Heinze T., Liebert T., Unconventional methods in cellulose functionalization, *Prog. Polym. Sci.*, **26**, 2001, 1689–1762.
3. Zhou J., Zhang L., Solubility of cellulose in sodium hydroxide/urea aqueous solution, *Polymer J.*, **32**, 2000, 866–870.
4. Heinze T., Liebert T., Klüfers F., Meister F., Carboxymethylation of cellulose in unconventional media, *Cellulose*, **6**, 1999, 153–165.
5. Dawsey T.R., Application and limitations of lithium chloride/*N*,*N*-dimethylacetamide in the homogeneous derivatization of cellulose, in *Cellulosic Polymers, Blends and Composite*, Ed.: Gilbert R.D., Hanser, Munich, 1994, pp. 157–171.
6. El Seoud O.A., Heinze T., Organic esters of cellulose: New perspectives for old polymers, in *Polysaccharides I, Structure, Characterization and Use*, *Advances in Polymer Science* **186**, Ed.: Heinze T., Springer Verlag, Berlin, Heidelberg, 2005, pp. 103–149.
7. Heinze T., Dicke R., Koschella A., Kull A.H., Klohr E.-A., Koch W., Effective preparation of cellulose derivatives in a new simple cellulose solvent, *Macromol. Chem. Phys.*, **201**, 2000, 627–631.
8. Köhler S., Heinze T., New solvents for cellulose: Dimethyl sulfoxide/ammonium fluorides, *Macromol. Biosci.*, **7**, 2007, 307–314.
9. Ass B.A.P., Frollini E., Heinze T., Studies on the homogeneous acetylation of cellulose in the novel solvent dimethyl sulfoxide/tetrabutylammonium fluoride trihydrate, *Macromol. Biosci.*, **4**, 2004, 1008–1013.
10. Sharma R.K., Fry J.L., Instability of anhydrous tetra-*n*-alkylammonium fluorides, *J. Org. Chem.*, **48**, 1983, 2112–2114.
11. Sun H., DiMagno S.G., Anhydrous tetrabutylammonium fluoride, *J. Am. Chem. Soc.*, **127**, 2005, 2050–2051.
12. Philipp B., Organic solvents for cellulose, *Polym. News*, **15**, 1990, 170–175.
13. Woodings C.R., The development of advanced cellulosic fibers, *Int. J. Biol. Macromol.*, **17**, 1995, 305–309.
14. O'Driscoll C., Spinning a stronger yarn, *Chem. Brit.*, **32**, 1996, 27–29.
15. Wendler F., Graneß G., Heinze T., Characterization of autocatalytic reactions in modified cellulose/NMMO solutions by thermal analysis and UV/VIS spectroscopy, *Cellulose*, **12**, 2005, 411–422.
16. Wendler F., Fortschritte bei der Characterisierung modifizierter Cellulose/*N*-Methylmorpholin-*N*-oxid-Lösungen. Ein Beitrag zur Herstellung funktionalisierter Celluloseformkörper, Ph.D. Thesis, Friedrich Schiller University of Jena, 2006.
17. Swatloski R.P., Spear S.K., Holbrey J.D., Rogers R.D., Dissolution of cellulose with ionic liquids, *J. Am. Chem. Soc.*, **124**, 2002, 4974–4975.
18. Schlufter K., Schmauder H.-P., Dorn S., Heinze T., Efficient homogeneous chemical modification of bacterial cellulose in the ionic liquid 1-*N*-butyl-3-methylimidazolium chloride, *Macromol. Rapid Comm.*, **27**, 2006, 1670–1676.
19. Husemann E., Siefert S., *N*-ethyl-pyridinium-chlorid als Lösungsmittel und Reaktionsmedium für Cellulose, *Makromol. Chem.*, **128**, 1969, 288–291.
20. Deus C., Friebolin H., Siefert E., Partially acetylated cellulose: Synthesis and determination of the substituent distribution via proton NMR spectroscopy, *Makromol. Chem.*, **192**, 1991, 75–83.
21. Wu J., Zhang J., Zhang H., He J., Ren Q., Guo M., Homogeneous acetylation of cellulose in a new ionic liquid, *Biomacromolecules*, **5**, 2004, 266–268.
22. Heinze T., Schwikal K., Barthel S., Ionic liquids as reaction medium in cellulose functionalization, *Macromol. Biosci.*, **5**, 2005, 520–525.
23. Barthel S., Heinze T., Acylation and carbanilation of cellulose in ionic liquids, *Green Chem.*, **8**, 2006, 301–306.
24. McCormick C.L., Lichatowich D.K., Pelezo J.A., Anderson K.W., Homogeneous solution reactions of cellulose, chitin, and other polysaccharides, *Modification of Natural Polymers*, ACS Symposium Series No. 121, 1980, pp. 371–380.
25. McCormick C.L., Callais P.A., Derivatization of cellulose in lithium chloride and *N*,*N*-dimethylacetamide solutions, *Polymer*, **28**, 1987, 2317–2323.
26. El Seoud O.A., Marson G.A., Ciacco G.T., Frollini E., An efficient, one-pot acylation of cellulose under homogeneous reaction conditions, *Macromol. Chem. Phys.*, **201**, 2000, 882–889.
27. Regiani A.M., Frollini E., Marson G.A., Arantes G.M., El Seoud O.A., Some aspects of acylation of cellulose under homogeneous solution conditions, *J. Polym. Sci. A: Polym. Chem.*, **37**, 1999, 1357–1363.
28. Ciacco G.T., Liebert T.F., Frollini E., Heinze T.J., Application of the solvent dimethyl sulfoxide/tetrabutyl-ammonium fluoride trihydrate as reaction medium for the homogeneous acylation of sisal cellulose, *Cellulose*, **10**, 2003, 125–132.
29. Heinze T., Schaller J., New water soluble cellulose esters synthesized by an effective acylation procedure, *Macromol. Chem. Phys.*, **201**, 2000, 1214–1218.
30. Sealey J.E., Frazier C.E., Samaranayake G., Glasser W.G., Novel cellulose derivatives. V. Synthesis and thermal properties of esters with trifluoroethoxy acetic acid, *J. Polym. Sci. B: Polym. Phys.*, **38**, 2000, 486–494.
31. Sealey J.E., Samaranayake G., Todd J.G., Glasser W.G., Novel cellulose derivatives. IV. Preparation and thermal analysis of waxy esters of cellulose, *J. Polym. Sci. B: Polym. Phys.*, **34**, 1996, 1613–1620.

32. Heinze T., Liebert T., Pfeiffer K., Hussain M.A., Unconventional cellulose esters: Synthesis, characterization and structure–property relations, *Cellulose*, **10**, 2003, 283–296.
33. Glasser W.G., Samaranayake G., Dumay M., Dave V., Novel cellulose derivatives. III. Thermal analysis of mixed esters with butyric and hexanoic acid, *J. Polym. Sci. B: Polym. Phys.*, **33**, 1995, 2045–2054.
34. Glasser W.G., Becker U., Todd J.G., Novel cellulose derivatives. Part VI. Preparation and thermal analysis of two novel cellulose esters with fluorine-containing substituents, *Carbohyd. Polym.*, **42**, 2000, 393–400.
35. Samaranayake G., Glasser W.G., Cellulose derivatives with low DS, 1. A novel acylation system, *Carbohyd. Polym.*, **22**, 1993, 1–7.
36. Zhang Z.B., McCormick C.L., Structopendant unsaturated cellulose esters via acylation in homogeneous lithium chloride/*N*,*N*-dimethylacetamide solutions, *J. Appl. Polym. Sci.*, **66**, 1997, 293–305.
37. Williamson S.L., McCormick C.L., Cellulose derivatives synthesized via isocyanate and activated ester pathways in homogeneous solutions of lithium chloride/*N*,*N*-dimethylacetamide, *J. Macromol. Sci. Pure Appl. Chem.*, **A35**, 1998, 1915–1927.
38. Staab H.A., Syntheses with heterocyclic amides (azolides), *Angew. Chem.*, **74**, 1962, 407–423.
39. Liebert T., Heinze T., Tailored cellulose esters: Synthesis and structure determination, *Biomacromolecules*, **6**, 2005, 333–340.
40. Hussain M.A., Liebert T., Heinze T., First report on a new esterification method for cellulose, *Polym. News*, **29**, 2004, 14–16.
41. Iwata T., Azuma J., Okamura K., Muramoto M., Chun B., Preparation and NMR assignments of cellulose mixed esters regioselectively substituted by acetyl and propanoyl groups, *Carbohyd. Res.*, **224**, 1992, 277–283.
42. Iwata T., Doi Y., Azuma J., Direct imaging of single crystals of regioselectively substituted cellulose esters by atomic force microscopy, *Macromolecules*, **30**, 1997, 6683–6684.
43. Tsunashima Y., Hattori K., Substituent distribution in cellulose acetates: Its control and the effect on structure formation in solution, *J. Colloid Interf. Sci.*, **228**, 2000, 279–286.
44. Tsunashima Y., Hattori K., Kawanishi H., Horii F., Regioselectively substituted 6-*O*- and 2,3-di-*O*-acetyl-6-*O*-triphenylmethylcellulose: Its chain dynamics and hydrophobic association in polar solvents, *Biomacromolecules*, **2**, 2001, 991–1000.
45. Rahn K., Diamantoglou M., Klemm D., Berghmans H., Heinze T., Homogeneous synthesis of cellulose *p*-toluenesulfonates in *N*,*N*-dimethylacetamide/LiCl solvent system, *Angew. Makromol. Chem.*, **238**, 1996, 143–163.
46. Koschella A., Leermann T., Brackhagen M., Heinze T., Study of sulfonic acid esters from $1 \rightarrow 4$-, $1 \rightarrow 3$-, and $1 \rightarrow 6$-linked polysaccharides, *J. Appl. Polym. Sci.*, **100**, 2006, 2142–2150.
47. Ishii T., Ishizu A., Nakano J., Chlorination of cellulose with methanesulfonyl chloride in *N*,*N*-dimethylformamide and chloral, *Carbohyd. Res.*, **59**, 1977, 155–163.
48. Sato T., Koizumi J., Ohno Y., Endo T., An improved procedure for the preparation of chlorinated cellulose with methanesulfonyl chloride in a dimethylformamide–chloral–pyridine mixture, *J. Polym. Sci. A: Polym. Chem.*, **28**, 1990, 2223–2227.
49. Furuhata K., Aoki N., Suzuki S., Sakamoto M., Saegusa Y., Nakamura S., Bromination of cellulose with tribromoimidazole, triphenylphosphine and imidazole under homogeneous conditions in lithium bromide–dimethylacetamide, *Carbohyd. Polym.*, **26**, 1995, 25–29.
50. Heinze T., Rahn K., New polymers from cellulose sulfonates, *J. Pulp Pap. Sci.*, **25**, 1999, 136–140.
51. Kasuya N., Iiyama K., Meshitsuka G., Ishizu A., Preparation of 6-deoxy-6-fluorocellulose, *Carbohyd. Res.*, **260**, 1994, 251–257.
52. Vigo T.L., Sachinvala N., Deoxycelluloses and related structures, *Polym. Adv. Technol.*, **10**, 1999, 311–320.
53. Siegmund G., Klemm D., Cellulose: Cellulose sulfonates: preparation, properties, subsequent reactions, *Polym. News*, **27**, 2002, 84–90.
54. Liu C., Baumann H., Exclusive and complete introduction of amino groups and their *N*-sulfo and *N*-carboxymethyl groups into the 6-position of cellulose without the use of protecting groups, *Carbohyd. Res.*, **337**, 2002, 1297–1307.
55. Heinze T., Koschella A., Brackhagen M., Engelhardt J., Nachtkamp K., Studies on non-natural deoxyammonium cellulose, *Macromol. Symp.*, **244**, 2006, 74–82.
56. Koschella A., Heinze T., Novel regioselectively 6-functionalized cationic cellulose polyelectrolytes prepared via cellulose sulfonates, *Macromol. Biosci.*, **1**, 2001, 178–184.
57. Tiller J., Berlin P., Klemm D., Novel matrices for biosensor application by structural design of redox-chromogenic aminocellulose esters, *J. Appl. Polym. Sci.*, **75**, 2000, 904–915.
58. Brandt L., Cellulose ethers, in *Ullmann's Encyclopedia of Industrial Chemistry*, Eds.: Gerhartz W., Yamamoto Y.S., Campbell F.T., Pfefferfcorn R., and Rounsaville J.F., VCH, Weinheim, 1986, **Volume A5**, 461ff.
59. Klemm D., Philipp B., Heinze T., Heinze U., *Comprehensive Cellulose Chemistry*, 1st Edition, Volumes 1 and 2, Wiley-VCH, Weinheim, 1998.
60. Klemm D., Heublein B., Fink H.-P., Bohn A., Cellulose: Fascinating biopolymer and sustainable raw material, *Angew. Chem. Int. Ed.*, **44**, 2005, 3358–3393.

61. Heinze T., Chemical functionalization of cellulose, in *Polysaccharides: Structural diversity and functional versatility*, Ed.: Dumitriu S., 2nd Edition, Marcel Dekker, New York, Basel, Hong Kong, 2004, pp. 551–590, Chapter 23.
62. Wagenknecht W., Regioselectively substituted cellulose derivatives by modification of commercial cellulose acetates, *Papier* (Darmstadt, Germany), **50**, 1996, 712–720.
63. Altaner C., Saake B., Puls J., Specificity of an *Aspergillus niger* esterase deacetylating cellulose acetate, *Cellulose*, **10**, 2003, 85–95.
64. Harkness B.R., Gray D.G., Preparation and chiroptical properties of tritylated cellulose derivatives, *Macromolecules*, **23**, 1990, 1452–1457.
65. Kondo T., Gray D.G., The preparation of *O*-methyl- and *O*-ethyl-cellulose having controlled distribution of substituents, *Carbohyd. Res.*, **220**, 1991, 173–183.
66. Kern H., Choi S.W., Wenz G., Heinrich J., Ehrhardt L., Mischnik P., Garidel P., Blume A., Synthesis, control of substitution pattern and phase transitions of 2,3-di-*O*-methylcellulose, *Carbohyd. Res.*, **326**, 2000, 67–79.
67. Kasuya N., Sawatari A., A simple and facile method for triphenylmethylation of cellulose in a solution of lithium chloride in *N*,*N*-dimethylacetamide, *Sen'i Gakkaishi*, **56**, 2000, 249–253.
68. Hagiwara L., Shiraishi N., Yokota T., Homogeneous tritylation of cellulose in a sulphur dioxide–diethylamine–dimethylsulfoxide medium, *J. Wood Chem. Technol.*, **1**, 1981, 93–109.
69. Camacho Gómez J.A., Erler U.W., Klemm D.O., 4-Methoxy substituted trityl groups in 6-*O* protection of cellulose: Homogeneous synthesis, characterization, detritylation, *Macromol. Chem. Phys.*, **197**, 1996, 953–964.
70. Pawlowski W.P., Gilbert R.D., Fornes R.E., Purrington S.T., The liquid-crystalline properties of selected cellulose derivatives, *J. Polym. Sci. B: Polym. Phys.*, **26**, 1988, 1101–1110.
71. Petzold K., Koschella A., Klemm D., Heublein B., Silylation of cellulose and starch – Selectivity, structure analysis, and subsequent reactions, *Cellulose*, **10**, 2003, 251–269.
72. Koschella A., Klemm D., Silylation of cellulose regiocontrolled by bulky reagents and dispersity in the reaction media, *Macromol. Symp.*, **120**, 1997, 115–125.
73. Mischnik P., Lange M., Gohdes M., Stein A., Petzold K., Trialkylsilyl derivatives of cyclomaltoheptaose, cellulose, and amylose: Rearrangement during methylation analysis, *Carbohyd. Res.*, **277**, 1995, 179–187.
74. Heinze T., Röttig K., Nehls I., Synthesis of 2,3-*O*-carboxymethylcellulose, *Macromol. Rapid Comm.*, **15**, 1994, 311–317.
75. Liu H.-Q., Zhang L.-N., Takaragi A., Miyamoto T., Water solubility of regioselectively 2,3-*O*-substituted carboxymethylcellulose, *Macromol. Rapid Comm.*, **18**, 1997, 921–925.
76. (a) Yue Z., Cowie J.M.G., Preparation and chiroptical properties of a regioselectively substituted cellulose ether with PEO side chains, *Macromolecules*, **35**, 2002, 6572–6577. (b) Schaller J., Heinze T., Studies on the synthesis of 2,3-*O*-hydroxyalkyl ethers of cellulose, *Macromol. Biosci.*, **5**, 2005, 58–63.
77. Hirrien M., Chevillard C., Desbrieres J., Axelos M.A.V., Rinaudo M., Thermal gelation of methyl celluloses: New evidence for understanding the gelation mechanism, *Polymer*, **39**, 1998, 6251.
78. Kondo T., Preparation of 6-*O*-alkylcelluloses, *Carbohyd. Res.*, **238**, 1993, 231–240.
79. Itagaki H., Tokai M., Kondo T., Physical gelation process for cellulose whose hydroxyl groups are regioselectively substituted by fluorescent groups, *Polymer*, **38**, 1997, 4201–4205.
80. Kondo T., Sawatari C., Manley R.S., Gray D.G., Characterization of hydrogen-bonding in cellulose synthetic polymer blend systems with regioselectively substituted methylcellulose, *Macromolecules*, **27**, 1994, 210–215.
81. Shin J.-H., Kondo T., Cellulosic blends with poly(acrylonitrile): Characterization of hydrogen bonds using regioselectively methylated cellulose derivatives, *Polymer*, **39**, 1998, 6899–6904.
82. Kondo T., Hydrogen bonds in regioselectively substituted cellulose derivatives, *J. Polym. Sci. B: Polym. Phys.*, **32**, 1994, 1229–1236.
83. Kondo T., The relationship between intramolecular hydrogen bonds and certain physical properties of regioselectively substituted cellulose derivatives, *J. Polym. Sci. B: Polym. Phys.*, **35**, 1997, 717–723.
84. Kasai W., Kuga S., Magoshi J., Kondo T., Compression behaviour of Langmuir–Blodgett monolayers of regioselectively substituted cellulose ethers with long alkyl side chains, *Langmuir*, **21**, 2005, 2323–2329.
85. Nojiri M., Kondo T., Application of regioselectively substituted methylcelluloses to characterize the reaction mechanism of cellulase, *Macromolecules*, **29**, 1996, 2392–2395.
86. Koschella A., Heinze T., Klemm D., First synthesis of 3-*O*-functionalized cellulose ethers via 2,6-di-*O*-protected silyl cellulose, *Macromol. Biosci.*, **1**, 2001, 49–54.
87. Petzold K., Klemm D., Heublein B., Burchard W., Savin G., Investigations on structure of regioselectively functionalized celluloses in solution exemplified by using 3-*O*-alkyl ethers and light scattering, *Cellulose*, **11**, 2004, 177–193.
88. Koschella A., Fenn D., Heinze T., Water soluble 3-mono-*O*-ethyl cellulose: Synthesis and characterization, *Polym. Bull.*, **57**, 2006, 33–41.
89. Illy N.G., Regioselektive Cellulosefunktionalisierung: Herstellung, Charakterisierung und Eigenschaftsuntersuchung von 3-*O*-Celluloseethern, *Diploma Thesis*, Friedrich Schiller University of Jena, Germany, 2006.

90. Dönges R., Nonionic cellulose ethers, *Br. Polym. J.*, **23**, 1990, 315–326.
91. Liebert T., Heinze T., Induced phase separation: A new synthesis concept in cellulose chemistry, in *Cellulose Derivatives: Modification, Characterisation, and Nanostructures*, Eds.: Heinze T.J. and Glasser W.G., ACS Symposium Series No. 688, American Chemical Society, Washington/DC, 1998, pp. 61–72.
92. Heinze T., Ionische Funktionspolymere aus Cellulose: Neue Synthesekonzepte, Strukturaufklärung und Eigenschaften, *Habilitation Thesis*, Friedrich Schiller University of Jena, Germany, 1997, Shaker Verlag, Aachen, 1998.
93. Heinze T., Erler U., Nehls I., Klemm D., Determination of the substituent pattern of heterogeneously and homogeneously synthesized carboxymethyl cellulose by using high-performance liquid chromatography, *Angew. Makromol. Chem.*, **215**, 1994, 93–106.
94. Reuben J., Conner H.T., Analysis of the ^{13}C NMR spectrum of hydrolyzed *O*-(carboxymethyl)cellulose: Monomer composition and substitution patterns, *Carbohyd. Res.*, **115**, 1983, 1–13.
95. Koschella A., Neue Funktionspolymere durch regioselektiv gesteuerte Synthesen aus Cellulose und NMR-spektroskopische Charakterisierung der unkonventionellen Substituentenmuster, *Ph.D. Thesis*, Friedrich Schiller University of Jena, Germany, 2000.
96. Ramos L.A., Frollini E., Heinze T., Carboxymethylation of cellulose in the new solvent dimethyl sulfoxide/tetrabutylammonium fluoride, *Carbohyd. Polym.*, **60**, 2005, 259–267.
97. Mann G., Kunze J., Loth F., Fink H.-P., Cellulose ethers with a block-like distribution of substituents by structure-selective derivatization of cellulose, *Polymer*, **39**, 1998, 3155–3165.
98. Fink H.-P., Dautzenberg G.H., Kunze J., Philipp B., The composition of alkali cellulose – A new concept, *Polymer*, **27**, 1986, 944–948.
99. Isogai A., Ishizu A., Nakano J., Preparation of tri-*O*-alkylcelluloses by the use of a nonaqueous cellulose solvent and their physical characteristics, *J. Appl. Polym.Sci.*, **31**, 1986, 341–352.
100. Petrus L., Gray D.G., BeMiller J.N., Homogeneous alkylation of cellulose in lithium chloride/dimethyl sulfoxide solvent with dimsyl sodium activation. A proposal for the mechanism of cellulose dissolution in lithium chloride/DMSO, *Carbohyd. Res.*, **268**, 1995, 319–323.
101. Kondo T., Gray D.G., Facile method for the preparation of tri-*O*-(alkyl)cellulose, *J. Appl. Polym. Sci.*, **45**, 1992, 417–423.
102. Takaragi A., Minoda M., Miyamoto T., Liu H.Q., Zhang L.N., Reaction characteristics of cellulose in the LiCl/1,3-dimethyl-2-imidazolidinone solvent system, *Cellulose*, **6**, 1999, 93–109.
103. Sachinvala N.D., Winsor D.L., Hamed O.A., Maskos K., Niemczura W.P., Tregre G.J., Glasser W.G., Bertoniere N.R., The physical and NMR characterizations of allyl- and crotylcelluloses, *J. Polym. Sci. A: Polym. Chem.*, **38**, 2000, 1889–1902.
104. Mormann W., Demeter J., Silylation of cellulose with hexamethyldisilazane in liquid ammonia – First examples of completely trimethylsilylated cellulose, *Macromolecules*, **32**, 1999, 1706–1710.
105. Stoehr T., Petzold K., Wolf B.A., Klemm D., Continuous polymer fractionation of polysaccharides using highly substituted trimethylsilylcellulose, *Macromol. Chem. Phys.*, **199**, 1998, 1895–1900.
106. Schaub M., Wenz G., Wegner G., Stein A., Klemm D., Ultrathin films of cellulose on silicon wafers, *Adv. Mater.*, **5**, 1993, 919–922.
107. Buchholz V., Wegner G., Stemme S., Oedberg L., Regeneration, derivatization, and utilization of cellulose in ultrathin films, *Adv. Mater.*, **8**, 1996, 399–402.
108. Löscher F., Ruckstuhl T., Seeger S., Ultrathin cellulose-based layers for detection of single antigen molecules, *Adv. Mater.*, **10**, 1998, 1005–1009.
109. Burger J., Kettenbach G., Klüfers P., Coordination equilibria in transition-metal based cellulose solvents, *Macromol. Symp.*, **99**, 1995, 113–126.
110. Fischer S., Thümmler K., Pfeiffer K., Liebert T., Heinze T., Evaluation of molten inorganic hydrates as reaction medium for the derivatization of cellulose, *Cellulose*, **9**, 2002, 293–300.
111. Zhou Q., Zhang L., Li M., Wu X., Gheng G., Homogeneous hydroxyethylation of cellulose in NaOH/urea aqueous solution, *Polym. Bull.*, **53**, 2005, 243–248.
112. Liebert T., Hänsch C., Heinze T., Click chemistry with polysaccharides, *Macromol. Rapid Comm.*, **27**, 2006, 208–213.
113. Philipp B., Nehls I., Wagenknecht W., Schnabelrauch M., Carbon-^{13} NMR spectroscopic study of the homogeneous sulfation of cellulose and xylan in the dinitrogen tetroxide-DMF system, *Carbohyd. Res.*, **164**, 1987, 107–116.
114. Schnabelrauch M., Vogt S., Klemm D., Nehls I., Philipp B., Readily hydrolyzable cellulose esters as intermediates for the regioselective derivatization of cellulose. 1. Synthesis and characterization of soluble, low-substituted cellulose formats, *Angew. Makromol. Chem.*, **198**, 1992, 155–164.
115. Liebert T., Schnabelrauch M., Klemm D., Erler U., Readily hydrolyzable cellulose esters as intermediates for the regioselective derivatization of cellulose. Part II. Soluble, highly substituted cellulose trifluoroacetates, *Cellulose*, **1**, 1994, 249–258.
116. Salin B.N., Cemeris M., Mironov D.P., Zatsepin A.G., Trifluoroacetic acid as solvent for the synthesis of cellulose esters. 1. Synthesis of triesters of cellulose and aliphatic carboxylic acids, *Koksnes Kimija*, **3**, 1991, 65–69.

117. Johnson D.C., Nicholson M.D., Haigh F.C., Dimethyl sulfoxide/paraformaldehyde: A nondegrading solvent for cellulose, *Appl. Polym. Symp.*, **28**, 1976, 931–994.
118. Saikia C.N., Dutta N.N., Borah M., Thermal behavior of some homogeneously esterified products of high α-cellulose pulps of fast growing plant species, *Thermochim. Acta*, **219**, 1993, 191–203.
119. Edgar K.J., Pecorini T.J., Glasser W.G., Long-chain cellulose esters: Preparation, properties, and perspective, in *Cellulose Derivatives: Modification, Characterisation, and Nanostructures*, Eds.: Heinze T.J. and Glasser W.G., ACS Symposium Series No. 688, American Chemical Society, Washington/DC, 1998, pp. 38–60.
120. Pawlowski W.P., Sankar S.S., Gilbert R.D., Fornes R.D., Synthesis and solid state carbon-13 NMR studies of some cellulose derivatives, *J. Polym. Sci. A: Polym. Chem.*, **25**, 1987, 3355–3362.