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Analytical Methods in Wood Chemistry, Pulping, and Papermaking

With 129 Figures and 36 Tables

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Preface

In its broadest sense, and according to the traditional conception, wood chemistry is a comprehensive discipline, ranging from fundamental studies to practical applications. The manifold constituents, located in different morphological regions in the wood, results in an extreme complexity of wood chemistry. Ever more sophisticated endeavors needing fundamental studies and advanced analytical methods are necessary in order to delve deeper into various problems in pulping and papermaking. Gradually, new, improved analytical methods, originally developed for research purposes, are currently replacing many of the old "routine" methods in practical applications.

Because of the expanse of the subject, an attempt to write a book of this size about analytical methods seems, perhaps, too ambitious. Of course, a whole book series of several volumes would be necessary to cover this topic completely. However, there is undoubtedly a need for a more condensed presentation which does not go into experimental details, but is limited to the basic principles of the analytical methods and illustrates their applications. The emphasis is on more advanced and potential methods, and particularly on those based on different types of spectroscopy and chromatography. The discussion is extended to the preparative stages since, particularly for isolation of samples, special techniques are often needed prior to the actual analysis. Background theory is included because several of the methods discussed are used today mainly for research purposes and are as yet seldom applied to practical problems in pulping and papermaking. However, as already pointed out, it is believed that these modern methods will eventually be used in actual practice.

The purpose of the first two chapters (Chaps. 1 and 2) of the book is to give basic information on the chemical composition and analytical techniques of wood, including references to the morphological location of the wood constituents. The next three chapters (Chaps. 3–5) are devoted to the analytical methods of wood components according to the main groups (carbohydrates, lignin, and extractives). Chapter 6 deals with the methods that can be used for the characterization of the chemical properties of wood and pulp fibers directly, without a previous separation of the components. Special emphasis is given to the characterization of the fiber surfaces. The methods applied to the analysis of pulping liquors, bleach liquors, and papermaking waters and effluents are described in Chapters 7, 8, and 9, respectively. Finally, the book ends with Chapter 10, which deals with methods for analysis of inorganic components in wood and pulps.

The chapters of this book have been written by scientists who are all specialists in the respective fields. We are fully aware, however, of the individual preferences in the choices and recommendations of a given analytical method. Sometimes it has been necessary to find a balance between the authors' and the editors' opinions. We hope, nevertheless, that this book fulfills the pertinent standards of homogeneity and objectivity.

The book is intended for a broad category of users including both teachers and students having wood chemistry or related disciplines in their program as well as chemists and engineers working either in research or industry.

We are deeply indebted to Professor T. E. Timell, Series Editor, who offered us the opportunity to write this book and continuously encouraged us during the often troublesome periods of writing. Professor Timell reviewed the manuscripts in detail and improved them with respect to both content and language. His advice and encouragement contributed decisively to the completion of this book.

We thank the Faculty of Mathematics and Natural Sciences, University of Jyväskylä, for financial assistance in the preparation of the illustrations and Arja Siirto for her help.

Finally, we would like to thank the personnel at Springer Verlag for their cooperation and skillful editorial work.

Helsinki
Jyväskylä
September 1998

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1 Chemical Composition of Wood and Pulps: Basic Constituents and Their Distribution

E. Sjöström and U. Westermark

1.1 Introduction

The chemical composition of wood and pulps is briefly reviewed in this chapter, with reference to the relevant analytical problems. The purpose is to give a background to the following chapters, which deal specifically and in more detail with the separation techniques and analytical methods in wood chemistry.

Although several sources of cellulose are available, wood obtained from forest trees is the predominant material for making pulp and paper. Forests thus form the basis for the pulp and paper industry.

Trees are seed-bearing plants (Spermatophytae), which are subdivided into two groups: gymnosperms (Gymnospermae), known as softwoods (conifers), and angiosperms (Angiospermae), known as hardwoods. In Europe, only relatively few tree species exist naturally compared with North America, where the variety is much greater. Despite the extreme diversity of, particularly, hardwood species on the earth, especially in tropical forests, only a few tree species are widely used in the pulp and paper industry. It is to be expected, however, that the raw-material basis will diversify in the future.

Wood is a complex and non-uniform material, in respect to both its anatomical and its chemical properties. It is made up of different kinds of cells, performing the necessary functions of mechanical support, water transport, and metabolism. In addition to the characteristic differences between the cell types, the chemical composition of wood substance differs in various parts of the tree; in stem, branches, tops, roots, and bark. A notable variation occurs in the radial direction (growth or annual rings) with alternating layers of earlywood (light-colored, porous) and latewood (darker, denser). The influence of the age of the tree should also be considered in this connection. Juvenile (core) wood, produced by the young vascular cambium, is deposited in the annual rings during the earliest 5–20 years of growth. A high proportion (50%) of juvenile wood can be present in fast-grown plantation trees. Especially in conifers, juvenile wood differs from mature (adult) wood with regard to its anatomical, chemical, and physical properties. Most of it consists of earlywood. It is frequently an inferior raw material for lumber, pulp, and paper.

Successively, as the tree grows older, the water transport in the inner part of the stem becomes restricted because of the formation of dead heartwood. When sapwood is transformed into heartwood, the tree begins to synthesize phenolic and other components, which are deposited in the heartwood

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region. These compounds are often, although not always, specific for heartwood and not present in sapwood. They also tend to be specific for each tree species.

Trees can form special types of tissues as a response to outer forces. When the normal conditions for growth are disturbed so that the tree is brought out from its equilibrium position, the tree begins to produce so-called reaction wood. This type of wood is known as compression wood in gymnosperms and as tension wood in angiosperms. Like juvenile wood, reaction wood differs from normal wood not only anatomically and physically but also chemically.

Only debarked stem wood is normally used for pulping. The final pulp is composed of various types of fibers, which in softwood pulps are derived from the tracheids and in hardwood pulps from the fibers and vessels. Compared with the native wood elements, the pulp fibers have been more or less modified, or even partially damaged, depending on the particular defibration (pulping) process used. The strength and other mechanical properties of the single fibers depend on both the physical state and the chemical composition of the cell walls. For interfiber bonding, necessary for strength in paper sheets, the surface properties and the flexibility of the fibers are important.

Wood cells are built up of an insoluble polymeric matrix of polysaccharides and lignin. Wood extractives, usually representing only a minor fraction, are composed of mostly low-molecular weight material, largely deposited outside the cell wall. They consist of an extremely complex mixture of more or less lipophilic components, soluble in organic solvents. In addition, water-soluble hydrophilic substances as well as inorganic salts are present in wood. Finally, trace amounts of nitrogenous derivatives, such as proteins, nucleic acids, and alkaloids are also present in the wood cell walls. However, methods for their isolation and identification are outside the scope of this book.

1.2 Gross Composition of Wood

Determination of the gross chemical composition of a given wood sample without considering the morphological distribution of the main groups or individual components, represents a common type of wood analysis. Many analyses can be made directly on the disintegrated wood sample. However, for studying the chemical structures and polymer properties of the wood constituents, a preceding isolation of them is necessary.

Classical wet-chemistry methods are still used in the analysis of wood even though many of them have been replaced by modern techniques including various forms of chromatography and spectroscopy. Usually, however, these methods must be combined with adequate pretreatment techniques or group separation procedures. Indeed, a selective fractionation of the

wood polymers and their breakdown into simpler moieties or monomeric units are necessary prestages, which often causes great problems or even overwhelming obstacles.

Typical values showing the chemical composition of common pulpwood species have been summarized in Table 1.1 (softwood) and Table 1.2 (hardwood).

1.2.1 Polysaccharides

Approximately two thirds of the wood substance is composed of polysaccharides (cellulose and various hemicelluloses). A rough estimate of the total content of polysaccharides can be obtained by subjecting extractive-free wood to an oxidative delignification (usually with sodium chlorite), after which the polysaccharide material (holocellulose) remains as a solid residue. However, a more reliable analysis is based on the separate determination of the polysaccharide constituents using chromatographic methods. The extractive-free wood sample is subjected to an acid hydrolysis (usually with sulfuric acid) after

Table 1.1. The main constituents of softwood in percent of dry wood weight; average values typical of common pulpwood species

Constituent	Normal wood	Compression wood
Cellulose	37–43	29–31
Galactoglucomannans	15–20	9–12
Arabinoglucuronoxylan	5–10	6–8
Galactan		9–11
Laricinan (1,3-glucan)		3–5
Lignin	25–33	37–40
Extractives	2–5	2–5

Table 1.2. The main constituents of hardwood in percent of dry wood weight; average values typical of common pulpwood species

Constituent	Normal wood	Tension wood
Cellulose	39–45	50–65
Glucuronoxylan	15–30	16–23
Glucomannan	2–5	2–4
Galactan		0–10
Lignin	20–25	16–20
Extractives	2–4	2–4

which the liberated monosaccharides are separated and quantified. Apart from the difficulties involved (nonspecific degradation of carbohydrates cannot be completely avoided and uronosidic bonds in xylan are cleaved only partially), the content of both cellulose and various hemicelluloses can be calculated fairly accurately on the basis of the chromatographic analysis of the monosaccharides in hydrolyzates. No simple methods are available, however, for the specific determination of uronic acid moieties.

The early methods of the 1950s for separation and analysis of the monosaccharides in wood hydrolyzates were based on paper chromatography, paper electrophoresis, and conventional column chromatography. Soon after introduction of gas chromatography (GC) in the early 1960s and after the discovery of converting the carbohydrates into volatile derivatives by per(trimethylsilyl)ation, GC became an effective tool in carbohydrate chemistry. Combination of GC with mass spectrometry (MS) and introduction of fused-silica capillary columns with a high separation efficiency in the 1980s, signified a great step forward. Silylation is still the most common derivatization method even if other derivatives have been introduced. For quantitative determination of monosaccharides in wood and pulp hydrolyzates, their GC separation as alditol acetates is very useful.

High-performance liquid chromatography (HPLC) is another effective separation method. In comparison with GC, it has the advantage that derivatization is generally not needed and its use is not restricted to monosaccharides and lower oligosaccharides. Often, however, the two methods are used in parallel.

In the structural studies of polysaccharides, methylation analysis and GC/MS of the methylated fragments as well as various spectroscopic methods (especially ^{1}H and ^{13}C NMR) are important.

Cellulose. In most pulpwood species, 40–45% of the dry substance is cellulose. The chemical structure of cellulose has been clarified in detail, but its supermolecular state and its polymer properties are not yet fully understood.

Cellulose is a linear homopolymer of β-D-glucopyranose units (in $^{4}C_{1}$ conformation) joined together with glycosidic bonds (Fig. 1.1). Because of the strong tendency for intra- and intermolecular hydrogen bonding, bundles of cellulose molecules aggregate to microfibrils, which form either highly ordered (crystalline) or less ordered (amorphous) regions. Microfibrils are further aggregated to fibrils and finally to cellulose fibers. The tight fiber structure created by hydrogen bonds results in the typical material properties of cellulose, such as high tensile strength and insolubility in most solvents.

X-ray and other diffraction methods have played a decisive role in the analysis of the crystalline structure of cellulose. Even though there are still contradictory opinions, the view that the chains of native cellulose are parallel has been generally accepted.

For isolation of cellulose from wood in a pure and undegraded form, needed for studying its polymer properties, no reasonably simple methods are avail-

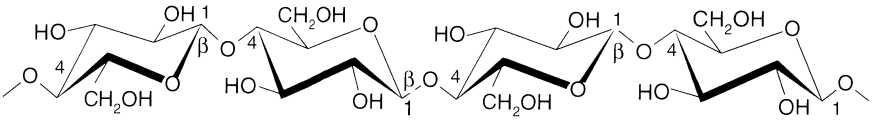


Fig. 1.1. A segment of cellulose chain that shows the conformation of the 1,4-linked β-D-glucopyranose units with equatorially oriented hydroxyl and hydroxymethyl groups

able. However, a direct nitration of wood yields undegraded cellulose trinitrate, which is soluble in organic solvents. Determination of its weight-average molecular weight ($\overline{M}_w$) by light-scattering, in addition to sedimentation-equilibrium (ultracentrifugation) measurements, indicate that the $\overline{DP}_w$ of native cellulose is of the order of 10000 and lower than that of cotton cellulose (about 15000).

Cellulose is soluble only in a few solvents, of which the most common are cupriethylenediamine (CED) and cadmiumethylenediamine (Cadoxen). Measurements of the molecular weights as well as other polymer properties of more or less degraded cellulose samples are regularly carried out using these solvents. The most common and convenient technique, suitable also for routine purposes, is based on viscosity measurements. Other, and more sophisticated, are the methods already mentioned above, and the osmometry giving the number-average molecular weight ($\overline{M}_n$), which also is obtained by a chemical determination of the number of reducing end groups. However, this method is rather inaccurate in the case of higher molecular weights.

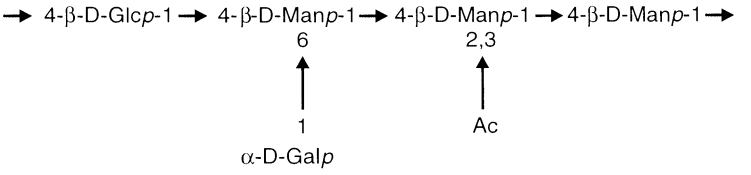
The polydispersity of native wood cellulose is probably quite low, which means that the $\overline{DP}_n$ and $\overline{DP}_w$ do not deviate much from each other. Measurements of its polymer properties indicate that cellulose in solution belongs to the group of randomly coiling polymers. The high viscosity and other polymer properties show that cellulose is a solvent-swollen polymer in contrast to lignin, which in solution occupies a compact structure.

Major Hemicelluloses. This group consists of various heteropolysaccharides and in most wood species their content is 20–30% of the dry wood weight. Hemicelluloses are usually isolated from holocellulose which is subjected to successive extractions with dimethyl sulfoxide and aqueous alkali. Gradient extractions using increasing alkali concentrations result in a rough separation of the hemicellulose components, which can be precipitated from the solution and further purified. However, degradation caused by the alkali cannot be completely avoided.

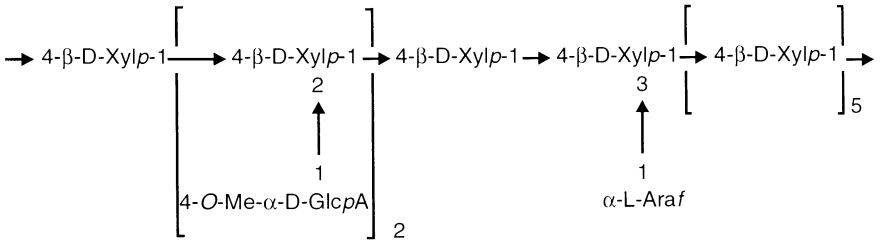
The hemicelluloses are deposited in the cell walls as matrix substances (except for arabinogalactan in larches, which is extracellular). In their native state the hemicelluloses are amorphous and their $\overline{DP}_w$ is of the order of 200–300. In addition to the principal monosaccharide building units

(D-glucose, D-mannose, D-galactose, D-xylose, L-arabinose, and L-rhamnose), uronic acid residues are present, of which 4-*O*-methyl-D-glucuronic acid predominates. The main hemicellulose constituents in wood can be classified as follows (Fig. 1.2).

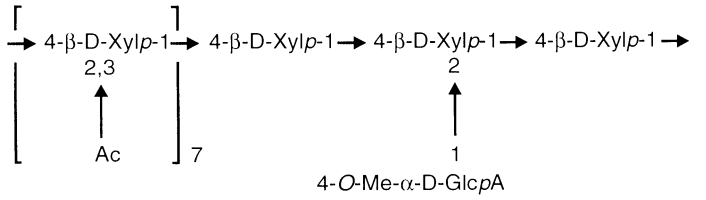
Galactoglucomannans (softwood constituents) are built up of a mainly linear backbone of (1 → 4)-linked and partially acetylated β-D-glucopyranose



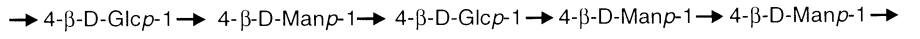
Galactoglucomannan (softwood)



Arabinoglucuronoxylan (softwood)

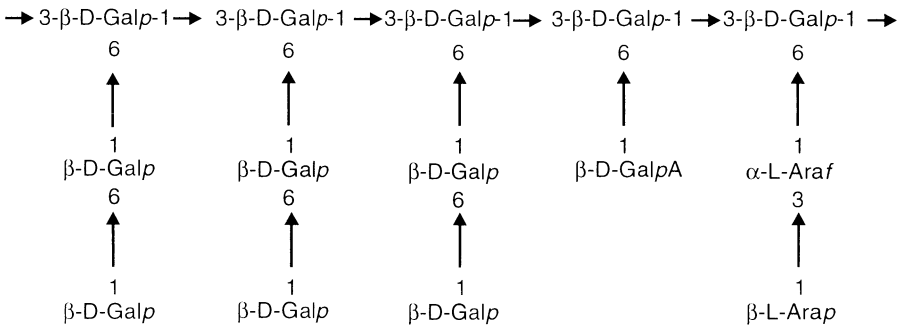


Glucuronoxylan (hardwood)

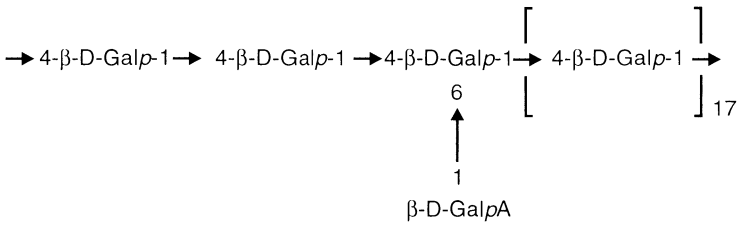


Glucomannan (hardwood)

Fig. 1.2. Abbreviated formulas of the most common wood hemicelluloses. For complete structures see, e.g., Sjöström (1993)



Arabinogalactan (larch wood)



Galactan (compression wood)

Fig. 1.2. *Continued*

and β-D-mannopyranose units, which are substituted at C-6 with a variable number of a single α-D-galactopyranose unit.

Glucomannan (hardwood constituent) has the same linear framework as galactoglucomannans, except that it is unsubstituted and has a higher glucose to mannose ratio.

Arabinoglucuronoxylan (softwood constituent) consists of a linear framework of (1 → 4)-linked β-D-xylopyranose units with branches of both 4-*O*-methyl-α-D-glucuronic acid and α-L-arabinofuranose. Unlike the hardwood xylan, no acetyl groups are present.

Glucuronoxylan (hardwood constituent) is composed of the same framework as the arabinoglucuronoxylan, but it contains much fewer uronic acid substituents. No arabinose units are present and the xylose residues are partially acetylated. A notable detail in both types of xylans is the presence of a dimeric segment consisting of an α-L-rhamnopyranose unit linked (1 → 2) to an α-D-galacturonic acid residue, which is attached to the 4-position of the reducing xylose end group.

Arabinogalactan, which occurs in the heartwood of larches (10–20%), consists of a backbone of (1 → 3)-linked β-D-galactopyranose residues, most of which carry a side chain attached to their 6-position. These consist of (1 → 6)-linked β-D-galactopyranose chains of variable length, an arabinose dimer, or a single unit of β-D-glucuronic acid. Unlike all other wood hemicelluloses, larch arabinogalactan is extracellular and can be extracted quantitatively from the heartwood with water.

Minor Polysaccharides. Wood also contains small amounts of miscellaneous polysaccharides (not all classified as hemicelluloses), including pectic substances (present only in primary cell walls), xyloglucan, starch, and, in compression wood, a (1 → 3)-β-D-glucan (laricinan). Pectic substances form a heterogeneous group including galacturonan (pectic acid), rhamnogalacturonan, arabinan, and galactans. *An acidic galactan* built up of (1 → 4)-linked β-D-galactopyranose units substituted at C-6 with a single α-D-galacturonic acid residue is a major hemicellulose in compression wood (Fig. 1.2).

1.2.2 Lignin

Lignin is deposited in the cell walls and the middle lamella as an encrusting substance, following the formation of the polysaccharides. Its physical role is to reinforce the wood structure. Often the plural form "lignins" is used because of the heterogeneity and structural varieties depending on the origin of the lignin. Lignin is an amorphous polymer, composed of hydroxylated phenylpropane units, which are linked together mainly with carbon-oxygen (ether) bonds but also with carbon-carbon bonds. Lignin exhibits a UV spectrum typical of aromatic compounds, a property that is useful for its quantitative determination. Various types of functional groups are present both in the aromatic ring and the propane side chain, affecting the properties and reactivity of lignin. There are also chemical linkages between the lignin and the hemicelluloses.

The content of lignin in wood can be determined gravimetrically and it varies within wide limits (20–33%) in different wood species and especially between softwood and hardwood. According to the classical Klason method, the extractive-free wood sample is first treated with a strong (72%) sulfuric acid solution, which is later diluted and heated to boiling. The polysaccharides are hydrolyzed and dissolved, whereas most of the lignin remains as a solid residue. The method is quantitative for softwood, but in the case of hardwood and pulp samples, the gravimetric values must be corrected by a spectrophotometric determination of the amount of lignin dissolved during the hydrolysis.

Despite many unsolved problems in the chemistry of lignin, the principal elements and the types of linkages are now known (Fig. 1.3). Improved

spectroscopic techniques have, in recent decades, resulted in more reliable and quantitative data concerning both the frequency of different bond types between the basic phenylpropane units and the nature and location of the various functional groups attached to these units. However, better methods for preparation of adequate samples and for selective isolation of native lignin from different morphological regions of the xylem are still needed.

As regards the determination of polymer properties of lignin, one great problem is its low solubility in most solvents. More data are available for modified and water-soluble lignin samples or derivatives, such as lignosulfonates and kraft lignin. For fractionation according to the molecular weight, size exclusion chromatography (SEC) is an effective method (Chap. 4). Vapor pressure osmometry, light scattering, and ultracentrifugation are more sophisticated research methods for determination of the molecular weights.

Since completely nondestructive methods are lacking for isolation of lignin from wood, no reliable molecular weight data are available for native lignin. For milled-wood lignin (MWL) samples of softwoods, the weight-average molecular weights ($\overline{M}_w$)are rather low, of the order of 20000. Lignosulfonates isolated from sulfite spent liquor can have molecular weights exceeding one million. The polydispersity of lignin seems to be about 2.5–3.0, much higher than that of cellulose.

Solutions of isolated lignin samples typically have a low viscosity, which means that the structure of the dissolved lignins is compact. The polymer

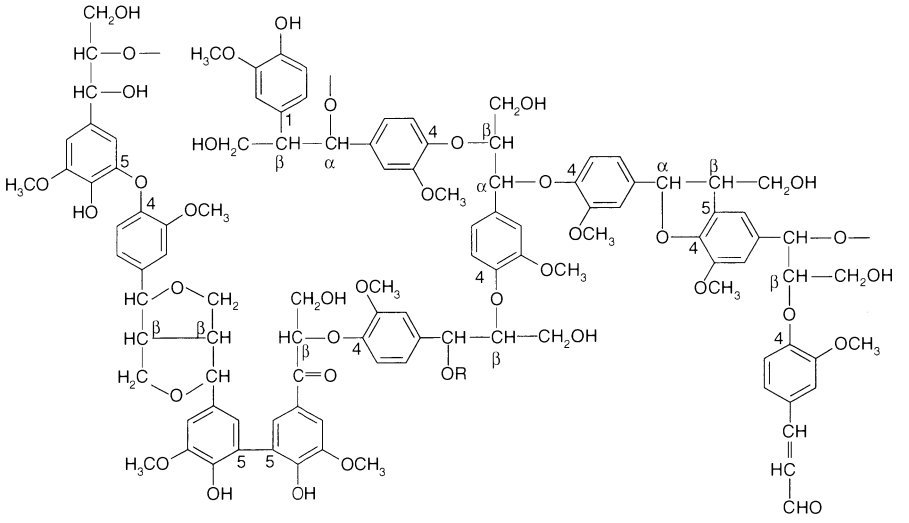


Fig. 1.3. Simplified representation of a segment of softwood lignin indicating the principal linkages between the phenylpropane units. R is H or a carbohydrate residue. The true proportions of the units and linkages cannot be seen from this formula but β-*O*-4 is the most dominant interunit linkage. The others are α-*O*-4 and 4-*O*-5 (less frequent ether linkages) and 5-5, β-5, β-1, and β-β (carbon linkages). For more information, see Chapter 4

properties of lignin are thus quite different from those of cellulose (see Sect. 1.2.1).

1.2.3 Extractives and Inorganic Components

A diversity of individual compounds belongs to the heterogeneous class of wood extractives. According to a rough classification, the main types are the following: terpenoids and steroids, fats (fatty acid glycerol esters), waxes (fatty acid esters of fatty alcohols, terpene alcohols, and sterols), and phenolic constituents including stilbenes, lignans, tannins, and flavonoids. Most of the compounds in these groups are lipophilic and soluble in organic solvents. Various hydrophilic (water-soluble) constituents, such as certain carbohydrates and inorganic components, are also present in wood.

The total content of lipophilic extractives in pulpwoods is usually 2–5% of the dry wood weight. According to the standard methods, the wood sample is extracted with an organic solvent under controlled conditions, after which the solvent is evaporated and the residue weighed. For separation and identification of the individual components in extractives, chromatographic methods are used, and since the extractives are mainly composed of low-molecular weight compounds, GC/MS is an ideal technique.

The content of inorganic components in pulpwoods is normally low, seldom exceeding 1% of the dry wood weight (determined as ash). The dominating cations are calcium, potassium, and magnesium, present mainly as carbonates, silicates, phosphates, and oxalates (predominantly in bark), or bound to the acid groups in wood. Iron and manganese occur in much smaller amounts and among the other transition metals, copper and cobalt are present only as traces. The analysis of the cations is usually made directly after wet ashing of the wood sample, using atomic absorption spectroscopy (AAS). More sophisticated methods include activation analysis and X-ray emission spectroscopy.

1.2.4 Basic Differences Between Softwoods and Hardwoods

Softwoods and hardwoods differ from each other not only with regard to their anatomy but also in their chemical composition. Some basic differences will be summarized in the following.

Hemicelluloses. In softwood, galactoglucomannans dominate (15–20%), whereas arabinoglucuronoxylan is the minor hemicellulose (5–10%). In hardwood, glucuronoxylan is the predominant hemicellulose (15–30%) and glucomannan is only a minor constituent (2–5%).

Lignin. The lignin content is usually considerably higher in softwoods (25–33%) than in hardwoods (20–25%). A striking structural difference between softwood and hardwood lignins is their different content of methoxyl groups. As a polymerization product of coniferyl alcohol, softwood lignin is built up of guaiacyl units (only one methoxyl group per phenylpropane unit), whereas hardwood lignin is a copolymer of coniferyl and sinapyl alcohols (two methoxyl groups per phenylpropane unit). Hardwood lignins contain fewer free phenolic hydroxyl groups but more free benzyl alcohol groups than softwood lignins. A third type of lignin is formed on polymerization of *p*-coumaryl alcohol. However, the resulting *p*-hydroxyphenyl lignin, typical of grasses, is found only in certain trees and tissues, usually as a copolymer with guaiacyl lignin.

Extractives. Softwood extractives are located both in the ray parenchyma cells (parenchyma resin) and in the resin canals, rich in pines (oleoresin). In hardwoods, the former type of extractives dominate because of the absence of resin canals. The parenchyma resin of both softwoods and hardwoods is composed of fats and waxes as well as triterpenoids and steroids. In addition, betulaprenols (polyterpenoids) are typical constituents of silver birch. Oleoresin is mainly composed of monoterpenoids and diterpenoids (including resin acids). Softwoods and hardwoods also differ from each other with respect to the composition of the phenolic extractives. It is noteworthy that even each species within various families and genera tends to produce its own, specific phenolic substances.

1.3 Distribution of Wood Constituents

The chemical composition of the wood substance in the stem differs from that of the other macroscopic parts of the tree (branches, knots, tops, and roots). Notable are also the variations within the same stem, especially in the radial direction (earlywood vs. latewood and sapwood vs. heartwood) as well as the differences between reaction and normal woods. The following discussion is restricted to stem wood, which is the predominant material used for pulping.

In the chemical characterization of different cell types, and particularly their carbohydrate composition, the studies so far made have been restricted to only a few wood species. Similarly, in investigations on the ultrastructural distribution of the wood constituents within the cell walls, usually only the most important cell types (tracheids and libriform fibers) have been considered.

1.3.1 Differences Between the Macroscopic Wood Parts and Fiber Types

The common wood analyses represent the overall composition of wood without any consideration of the different cell types. In most coniferous pulpwoods, the predominant cells are the axial tracheids (90–95%). In hardwoods, libriform fibers, fiber tracheids, and vessels dominate (70–80%). The composition of these main cell types is not far from the overall wood composition. However, fiber fractionation is necessary prior to any analysis of the composition of the minor cell types, such as the ray cells. Because the ray parenchyma cells and the ray tracheids present in some softwoods are of the same dimensions, the values reported usually include both of these cell types.

As regards the overall composition of coniferous wood, one difference is that latewood contains more cellulose and galactoglucomannans but less arabinoglucuronoxylan and lignin than earlywood. Typical of heartwood is its high content of extractives and the presence of many phenolic constituents not occurring in sapwood.

Compression wood differs from normal softwood especially in its very high lignin content (37–40%, Table 1.1). Furthermore, the content of methoxyl groups in compression wood is considerably lower than that in normal coniferous wood because it has been formed by copolymerization of coniferyl and *p*-coumaryl alcohols. There are also differences in the carbohydrate composition. The contents of both cellulose and galactoglucomannans in compression wood are lower, whereas the arabinoglucuronoxylan content is about the same as normal wood. An acidic galactan is a major hemicellulose in compression wood (≈10%). In addition, laricinan is a polysaccharide present only in compression wood, albeit in small amounts.

Tension wood also differs from normal hardwood (Table 1.2). It is noteworthy that the tension wood fibers contain an additional cell wall layer, the so-called gelatinous layer (G layer), which consists of only cellulose. Because of this layer, the cellulose content is higher and the lignin content lower in tension wood than in normal wood. Some hardwood species also contain an acidic, highly complex galactan.

The composition of the ray parenchyma cells differs significantly from that of the tracheids and fibers. The fat and wax components in both softwoods and hardwoods are located in the former cells. Their lignin content is higher than that of the tracheids and fibers. The ray cells of both softwoods and hardwoods contain much more xylan and correspondingly less (galacto)glucomannans than the other cells.

1.3.2 Composition of Cell Walls

The cell wall layers can be studied in situ by microscopic techniques, often in combination with spectroscopic methods. For a direct analysis of the cell wall

layers, special methods are needed for their separation. Largely because of the extreme difficulty of these separations, quantitative data for the composition of various cell wall layers are limited, especially with respect to their polysaccharides. The distribution profile also varies, depending on the origin and type of the wood (normal wood vs. reaction wood, earlywood vs. latewood, etc.). Only some principal features of the main fiber types of normal wood are considered in the following (Fig. 1.4).

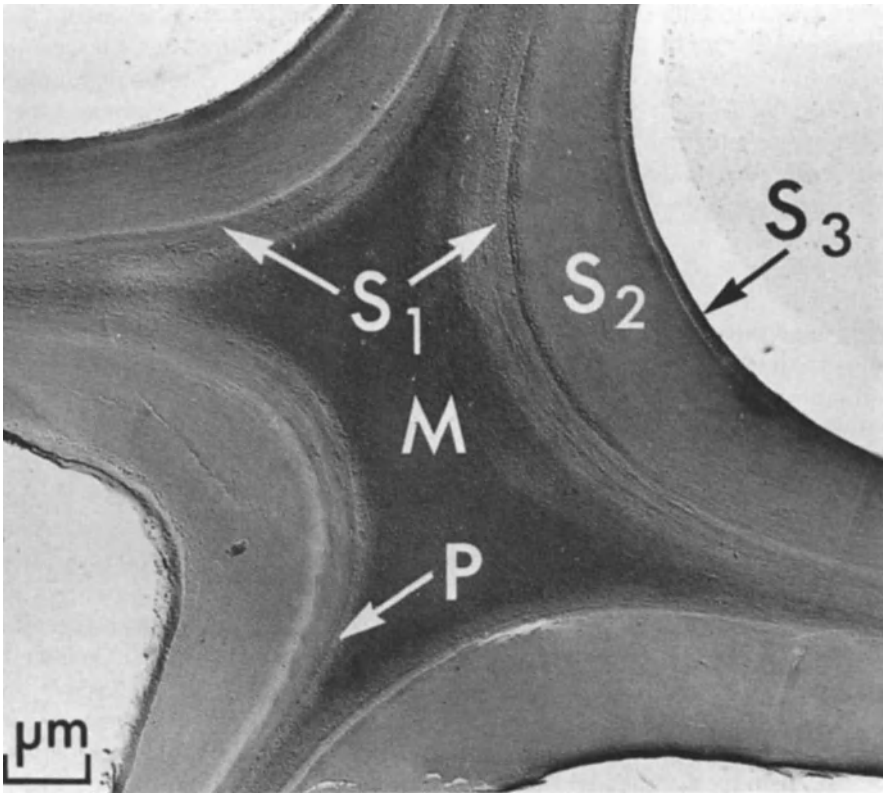
Polysaccharides. The first basic studies on the distribution of polysaccharides within the wood cell walls were made in the late 1950s, when tracheids and fibers were separated according to their age into fractions representing various stages of development. After acid hydrolysis, the resulting monosaccharides in each fraction were separated and analyzed by paper chromatography. Later approaches include the use of various microscopic techniques as well as fractionation methods after disintegration of the fiber material.

It is evident from these studies that in both softwoods and hardwoods the pectic polysaccharides occur in the M + P layer. However, views concerning the distribution of the main polysaccharides in the secondary wall layers are still conflicting. According to the earlier studies, in softwoods (spruce and pine), the cellulose concentration is highest in S_1 and S_2, while galactoglucomannans reach their highest concentration in S_2. The S_3 layer has a considerably higher content of xylan that S_1 and S_2. Later studies, based on fractionation, do not agree with these findings, and it has been reported that the concentrations of both galactoglucomannans and arabinoglucuronoxylan are even throughout the cell wall.

In hardwood (birch) the cellulose concentration seems to be highest in the inner part of S_2 and in S_3, whereas that of the glucuronoxylan is highest in S_1 and the outer portion of S_2.

Lignin. UV spectrophotometry in combination with UV microscopy is a useful and common method for studying the distribution of lignin in the cell wall and middle lamella. Other useful techniques for this purpose include energy-dispersive X-ray analysis (EDXA) in conjunction with scanning (SEM) and transmission (TEM) electron microscopy, a method recently improved by substituting mercurization for the nonspecific bromination step. TEM can also be used for examining the lignin skeletons remaining after the polysaccharides in wood have been removed by strong acids or fungal enzymes. After the lignin has been oxidized and stained by permanganate, its location can be accurately determined by TEM.

In softwood tracheids, the lignin concentration is high in the intercellular layer (M + P) and especially at the cell corners. In the secondary wall, the lignin concentration is much lower, and the distribution profile through its various layers seems to be rather uniform (albeit with a higher lignin concentration in the S_3 in some conifer species). Because of the thickness of S_2, 70–75% of the total lignin is located in the secondary wall of the tracheids.



Layer	Cellulose	GGM[a]	Xylan	Other polys.[b]	Lignin
M + P	0.9	0.1	0.3	1.6	8.5
S_1	2.6	0.9	0.9	Traces	1.4
S_2	35.7	14.9	9.0	Traces	19.8
S_3	1.5	0.6	0.4	Traces	0.9
Σ	40.7	16.5	10.6	1.6	30.6

[a] Galactoglucomannan. [b] Various polysaccharides, mainly pectic substances.

Fig. 1.4. Transverse section of softwood tracheids (earlywood of tamarack *Larix laricina*). The different regions are the middle lamella *M*, the primary wall *P*, and the outer S_1, middle S_2, and inner S_3 layers of the secondary wall. Scale 1 µm. Transmission electron micrograph. Courtesy of Dr. T. E. Timell. Reprinted from Sjöström (1993). *Below* are shown the proportions of the main cell wall constituents (*polysaccharides and lignin*) in the cell walls of softwood tracheids (% of the total amount). These are only approximate values, calculated for a given average thickness of the cell wall layers, and assuming a certain distribution profile of the constituents. The true values may considerably deviate from these data and they vary depending on the origin of the fibers

In hardwood fibers, the concentration of lignin is also highest in the middle lamella, and especially at the cell corners and the major portion of the total lignin is present in the secondary wall. When considering the whole wood, including fibers, vessels, and ray cells, the lignin present in the secondary walls of these elements accounts for nearly 80% of the total lignin in the wood. Both the ray parenchyma cells and the vessels have a higher lignin content (30–35%) than the fibers (20–25%).

Considerable, albeit partly conflicting information, is now available concerning the structural variations of lignin with respect to its morphological location. Without doubt, however, the lignin in the secondary wall of softwoods is of the guaiacyl type, but it has been reported that the middle lamella lignin instead is built up of both guaiacyl and *p*-hydroxyphenyl units, like the lignin in compression wood. However, there are other studies that do not agree with these findings. Nevertheless, its seems that the middle lamella lignin has more carbon-carbon bonds and is thus more condensed than the secondary wall lignin. This is perhaps one reason for the relatively low reactivity of the middle lamella lignin in pulping.

In the hardwoods, the lignin in the secondary wall of the fibers and the ray parenchyma cells is largely of the syringyl type, while that in the vessel wall is of the guaiacyl type. The vessel middle lamella lignin is also of the guaiacyl type, whereas a guaiacyl-syringyl lignin dominates in the fiber middle lamella.

1.4 Composition of Pulps

A wide range of commercial paper-grade pulps are produced for different end uses with a variable chemical composition. The following discussion will be limited to some general comments focusing only on typical features of the main pulp types.

Even though basically the same methods are applied in the analysis of wood and pulps, special techniques are often needed for the latter. One important question concerns the location and DP-distribution of the polysaccharides in the different morphological regions of the pulp fibers. However, because of experimental difficulties, only sparse information is available. Furthermore, characterization of the residual lignin in pulps and its association with the carbohydrates needs more attention.

1.4.1 Chemical Pulps

Polysaccharides. Cellulose and hemicelluloses are the main constituents of bleached chemical pulps. Their average $\overline{DP}_w$ is typically within the limits of

2000–4000 and 50–100, respectively. A large part of the original wood hemicelluloses are degraded and dissolved during pulping (30–70%), but most of the cellulose survives (≈90% yield for kraft and nearly 100% for acid sulfite pulping). With adequate bleaching conditions the carbohydrate losses are small. Typical data for the carbohydrate composition of the usual chemical pulp types are collected in Table 1.3.

The remaining hemicelluloses in softwood kraft pulps are composed of glucomannan and arabinoglucuronoxylan. Compared with the respective hemicellulose constituents in wood, both the acetyl groups and almost all of the galactose substituents are absent in the pulp glucomannan, and the number of glucuronic acid and arabinose side chains in the xylan is much reduced. Because of readsorption of xylan from the pulping liquor onto the pulp, kraft fibers have a characteristically high concentration of xylan on their surface. In hardwood kraft pulps, deacetylated glucuronoxylan (with much fewer acid side chains than in the original wood xylan) is the major hemicellulose constituent.

In softwood sulfite pulps the hemicelluloses are glucomannan (practically completely deacetylated during acid sulfite pulping) and glucuronoxylan. No galactose and arabinose substituents are present. The content of glucuronic acid groups in sulfite pulp xylan is higher than that in kraft pulp xylan. Glucuronoxylan is the major hemicellulose constituent in hardwood sulfite pulps, but its content is much lower than in hardwood kraft pulps.

Two-stage sulfite and polysulfide kraft pulps represent modified and less common softwood pulp types. Characteristically they have a high glucomannan content.

Lignin. Indirect determinations, based on oxidation and titration, are commonly applied for the quantitative determination of the residual lignin in the unbleached pulps (kappa number or chlorine number). The lignin content of pulps subjected for bleaching is rather low (2–5%) and only traces of it (<0.1%) are present in fully bleached pulps. For direct analysis of small

Table 1.3. Examples of typical carbohydrate compositions of common bleached pulps (relative percents)

Pulp type	Monosaccharides (analyzed)/Polysaccharides (calculated)					
	Glucose	Mannose	Xylose	Cellulose	Glucomannan	Xylan
Pine kraft[a]	83.2	6.4	10.4	81.3	8.0	10.7
Birch kraft	68.8	1.2	30.0	67.5	1.9	30.6
Spruce sulfite	85.3	7.0	7.7	82.9	8.8	8.3
Pine two-stage sulfite	79.7	13.6	6.7	74.1	18.3	7.6

[a] Small amounts of arabinose (<1%) are also present in softwood kraft pulps.

amounts of lignin, UV spectrophotometry can be applied after dissolution of the pulp sample in a proper solvent (acetyl bromide or Cadoxen).

Special isolation and analytical methods are needed for chemical characterization of the residual lignin. It can be degraded to smaller fragments by more or less specific oxidants, followed by chromatographic separation and identification of the fragments.

Extractives and Inorganic Components. Tall oil and turpentine are valuable kraft pulping by-products, but residues of extractives in pulps disturb the bleaching process and cause quality problems in the final pulp and paper. The pulp extractives are composed of a complex mixture of extremely lipophilic and sparingly soluble components of the original wood extractives as well as their reaction and polymerization products. Special techniques are necessary for their analysis and identification.

The major inorganic constituents in pulp originate from the pulping and bleaching chemicals and the process water. The other inorganics, present in ppm quantities, include compounds of iron, manganese, copper, and cobalt, derived from both the wood and the processing equipment. The ions of these metals are detrimental to the bleaching process because of their ability to generate carbohydrate-degrading radicals. Furthermore, they, as well as other cationic impurities, can be harmful in many final products, for example, high-quality printing papers, photocopying papers, foodboard, and electrical insulating papers.

Functional Groups. Carbonyl and carboxyl groups present in pulps are derived from wood and are additionally generated during pulping and bleaching operations. They are harmful because they contribute to the yellowing tendency (color reversion) of the paper, but no practical means exist for their elimination or transformation into harmless derivatives. The existence of carbonyl groups must also be considered in connection with the determination of pulp viscosity, since they will generate alkali-labile sites in cellulose. As a result of depolymerization in the alkaline cellulose solvents, erroneous viscosity values are obtained.

During bleaching, various positions in the polysaccharides are susceptible to an oxidative attack leading to the formation of carbonyl and carboxyl groups. The quantitative determination of the total content of carbonyl groups in pulps is usually based on their ability to form stable derivatives with certain reagents, for example, with phenylhydrazin. Even if carboxyl groups are generated during bleaching, the majority of them in most pulp types originate from the 4-*O*-methylglucuronic acid groups in the wood xylan. Numerous methods have been introduced for the quantitative determination of the carboxyl groups in pulps, but most of them are based on ion exchange.

In addition to the carboxyl groups in polysaccharides, the residual pulp lignin in unbleached sulfite pulps contains sulfonic acid groups. Even though the former are much weaker acids than the latter, they cannot be distinguished

from each other directly in the pulp by the usual ion exchange-titration procedures. Specific methods have been developed for their separate determination. Carboxylic acid groups and phenolic hydroxyl groups are also formed in the lignin residue during kraft pulping. Because of the low lignin content, however, their contribution to the total number of functional groups in unbleached chemical pulps is small.

Toxic Components. Today, elementary chlorine has been almost entirely eliminated as a bleaching agent at least in Nordic countries, but chlorine dioxide is still in widespread use. Since even traces of some chlorinated organic compounds can be extremely toxic, very sensitive analyses, based on mass spectrometry, have been developed for detection of them as well as other potentially harmful components in pulps.

1.4.2 Mechanical and Chemimechanical Pulps

Stone groundwood (SGW), pressure groundwood (PGW), refiner mechanical (RMP), and thermomechanical pulps (TMP) are the main types of mechanical pulps. Mechanical defibration of wood or chips results in only small material losses, and the gross composition of the resulting pulps differs only slightly from that of the original wood. However, the fiber structure is more or less damaged, and the pulps are usually characterized by fiber fractionation, followed by analysis of the fractions.

The production processes of chemimechanical pulps (CMP) normally involve a pretreatment of the wood chips with alkaline solutions of sodium sulfite at elevated temperatures. Chemithermomechanical pulps (CTMP) belong to this category. The yield is somewhat lower than for TMP, but the fibers are less damaged. In the pretreatment stage, sulfonic acid groups are introduced into the lignin. Because of the presence of these groups, the hydrophilicity and swelling of the lignin are increased, resulting in an easier defibration and in improved fiber bonding. Analytical methods for the determination of the sulfonic acid groups are important in characterization of chemimechanical pulps.

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Sjöström E 1993 Wood chemistry. Fundamentals and applications, 2nd edn. Academic Press, San Diego, 293 pp

Timell T 1986 Compression wood in gymnosperms, vol 1. Springer, Berlin Heidelberg New York, 706 pp

[1] Of the vast literature, the reader is referred only to the following textbooks of which proper parts and references can be selected for further reading.

2 Separation and Analysis of Wood Constituents with Respect to Their Morphological Location

U. Westermark and E. Sjöström

2.1 Introduction

Because of the heterogeneous nature of wood it is important that the material selected for analysis is of known origin. An incomplete characterization of the wood sample before analysis can lead to unreliable results because of contamination with tissues not typical of the expected normal wood. Guidelines for the selection of wood material are given in the first part of this chapter. The discussion is limited to stem wood.

Tracheids in softwoods and libriform fibers and fiber tracheids in hardwoods form the basis of paper, although the other cell types (ray cells and vessels) also contribute to its properties. Methods for isolation of the latter cell types from wood and pulps are therefore also discussed in this chapter.

Even if wood and pulps are usually considered as a bulk material, the main reactions and changes during pulping are taking place on the ultrastructural level, inside the fibers. In spite of this, most pulping studies have been made with whole wood and the ultrastructural phenomena have been neglected. One reason for this has been the experimental difficulties associated with the isolation of reasonable amounts of morphologically specified material. The existing methods for the analysis and separation of middle lamella and cell wall layers will be described in the last part of this chapter.

2.2 Separation of Macroscopic Wood Parts

2.2.1 Normal Wood

The proportions of juvenile wood to mature wood, earlywood to latewood, and heartwood to sapwood are important for the properties of the entire wood. In order to obtain a rough idea of these proportions, the stem should first be characterized with respect to width and age. The age of a certain annual ring is obtained by counting the annual rings (earlywood + latewood) from the center of the stem. The annual rings are visible to the eye as alternating light (earlywood) and dark (latewood) rings in most wood species from the temperate zone. For more detailed studies, the different tissues in wood can be isolated and characterized. Isolation of earlywood and latewood as well as other tissues in normal wood can best be made manually with a razor blade.

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Juvenile Wood. As already mentioned in Chapter 1, juvenile wood is formed by the young cambium and deposited in the first 5–20 annual rings (Abel-Gadir and Krahmer 1993). The best way to differentiate between juvenile and mature wood is to look at the annual rings and to measure the density of the wood. Juvenile wood has a lower density than the mature wood and its growth rings are often wide with a high proportion of earlywood. Juvenile wood can also be contaminated with relatively large amounts of compression wood.

Heartwood. The center of the stem is occupied by heartwood, which is usually, but not in all species, discernible because of its darker color in comparison with sapwood. However, the borderline between heartwood and sapwood is sometimes not readily distinguishable. When compared with softwood, characteristics of heartwood are the phenolic components, a lower moisture content, and a much lower permeability. Several staining and color reactions, mainly based on the reactions of phenolic extractives with certain reagents (e.g., sulfanilic acid and sodium nitrite), are available for the detection of heartwood (Hillis 1987). A simple way to distinguish heartwood in species where the color difference is small, is to polish the surface of the wood surface and then take a xerox at highest possible contrast. Heartwood will then appear as a light circle at the center of the stem (Yang 1987).

2.2.2 Compression Wood

Even if systematic studies are rather limited, there are indications that considerable quantities of compression wood can sometimes be present in pulpwood. Characteristic features indicating the presence of compression wood are the shape of the stem and the reddish-brown color of the wood (Timell 1986). An eccentric stem cross section is a typical sign of the presence of compression wood, although there are other reasons for this type of growth. Compression wood can also occur in completely symmetrical stems. Its presence can be verified by cutting the wood transversely into slices of 2.5–3.5 mm width and inspecting them under transmitted light, preferably with a microscope. Compression wood appears opaque whereas normal wood is transparent. This method is especially suitable for detection of small deposits of compression wood.

Microscopic examination reveals several distinctive features in the anatomical structure of compression wood. For example, the tracheids in compression wood are shorter than those in normal wood and their walls are relatively thick with a characteristic rounded outline. The cell corners are often unlignified and open with an intercellular space. Fully developed compression wood has helically oriented cavities in the S_2 layer and lacks an S_3 layer (Timell 1986).

When the purpose is to collect pure samples of normal wood without any contamination of abnormal wood, it should be noted that there is on the

opposite side of compression wood a special tissue, termed opposite wood. Because the properties of opposite wood differ from that of normal wood, this tissue should also be excluded.

When compression wood samples are to be isolated, it is best to choose a leaning or crooked stem. The compression wood can be localized by its reddish-brown color and opacity to transmitted light as mentioned above. In addition, when drying, compression wood shrinks more in the axial direction than normal wood. This means that compression wood sinks under the surface of normal wood on a cross section and for that reason it can be separated by hand with a scalpel or razor blade (Timell 1986).

2.2.3 Tension Wood

Eccentric stems in hardwoods typically indicate the presence of tension wood, although it can occur in perfectly straight stems as well. Many hardwood species lack the ability to form tension wood. Tension wood is more difficult to detect directly on the cross section of the stem than compression wood, since it often occurs mixed with normal wood. In some wood species, tension wood occurs in concentrated areas, but in many species it is present as discrete bands, predominantly in the earlywood. Tension wood is often visible to the naked eye because of its luster. Several staining techniques are available for detection and localization of tension wood by microscopy. Typical staining chemicals are zinc chloride combined with iodine, safranine, toluidine blue, and fluorescent stains used in combination with UV light (Casperson 1936; Mia 1968; Timell 1969).

2.3 Separation of Wood and Pulp Tissues

In addition to the fibers and other types of native wood cells, mechanical and high-yield pulps typically contain so-called fines. The fraction of fines is usually defined as the material passing a 200-mesh (0.074 mm) sieve. Ray cells and, in hardwoods, broken vessels, are typical components of fines. This section describes methods for isolation of fines, ray cells, and vessels from wood and pulps. Figure 2.1 illustrates the principle of a fractionation procedure.

2.3.1 Isolation of Fines from Pulps

Because fines significantly contribute to paper properties, their isolation is an important task in the characterization of pulps. The fraction of fines in

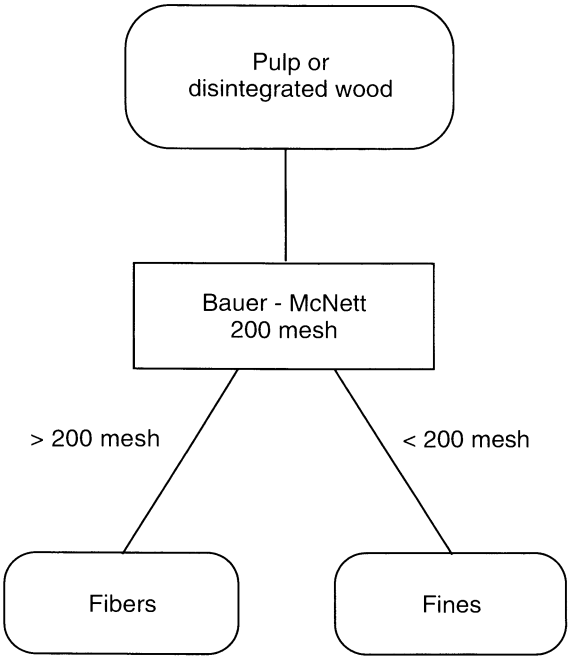

Fig. 2.1. Bauer-McNett fractionation of fines from pulps and disintegrated wood material. (Westermark 1985)

mechanical and high-yield pulps is heterogeneous and includes ray cells, crushed and broken fibers, and debris from the middle lamella and the cell wall. Bauer-McNett fractionation is a standard technique for separation of fines in pulps. A set of metal sieves, usually 30, 50, 100, and 200 mesh (corresponding to openings of 0.595, 0.297, 0.149, and 0.074 mm), is connected in series and the pulp is fractionated in several runs ($\approx$10 g for each run) to give sufficient amounts of fines ($<$200 mesh). The fine material is transported through the system in the water stream. The drawback is that the concentration of the suspension is very low. Instead of filtration, which is usually difficult, the water can be removed by centrifugation (relatively high G forces are needed for collection of the smallest particles). Lyophilization (freeze drying) or vacuum evaporation are alternative techniques, but the material that is dissolved during fractionation will then also be included or carried over to the concentrate.

Further fractionation of fines can be carried out by means of simple sieves, for example, of the type shown in Fig. 2.2. A jar equipped with nylon webs of different mesh number is soaked in a small volume of water for efficient washing out of small particles. This small scale sieving is useful as it gives efficient sieving in small volumes of water.

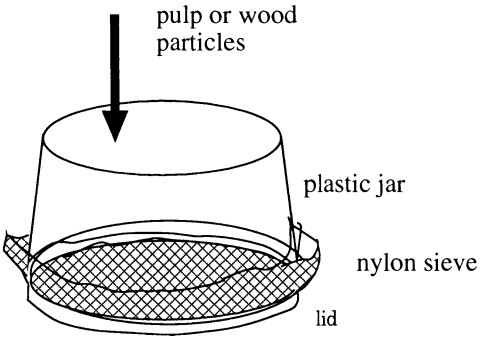

Fig. 2.2. A simple device for small-scale sieving

2.3.2 Isolation of Ray Cells from Wood and Pulps

Because the ray cells are short, they can be separated from the fibers by screening. Ray cells in pulps are present in a fraction passing a 50-μm sieve or a 200-mesh Bauer-McNett sieve. Fine fractions from disintegrated wood or high yield pulps contain cell debris of the same dimensions as the ray cells. Ray tracheids are, however, heavy and compact cells that can be separated from the fine fraction in a small laboratory hydrocyclone. According to the fractionation scheme for middle lamella, the ray cells are separated from the hydrocyclone reject (Sect. 2.4.4). Highly purified ray cell fractions can be obtained by manual separation (Fig. 2.3). A dilute fraction of fines, enriched with ray cells, is poured onto a watch glass which is then rotated slowly. The ray cells are gradually concentrated in the middle of the glass, whereas the debris remains at the periphery. This procedure should preferably be carried out under a stereo microscope at low magnification and repeated until a pure fraction is obtained (Westermark 1985). Ray cells from chemical pulps or holocellulose samples can be isolated simply by taking a fraction that has passed a 50-μm screen but is retained on a 20-μm screen (Perilä 1961; Hoffmann and Timell 1972; Westermark and Capretti 1988).

2.3.3 Isolation of Vessels from Wood and Pulps

Vessels are wide and short and their isolation directly from wood is difficult. They are usually interspersed between fibers and libriform cells in the wood tissue. The thin-walled vessels usually break into small pieces when the material is disintegrated or defiberized and they are hence difficult to collect. Thermomechanical pulps (TMP) that have been produced using a low energy input and a low temperature can, however, contain some whole vessels. Bauer-McNett fractionation is therefore possible for isolation of vessels from this type of pulp.

Fig. 2.3. Ray cells from Norway spruce (*Picea abies*) isolated from disintegrated wood material. Scanning electron micrograph

The vessels are enriched in a 65-mesh fraction (Marton et al. 1979). When a similar procedure as described in Section 2.4.4 is applied, the vessel fragments can be enriched in a fraction of $<80\,\mu m$ and $>50\,\mu m$ (Hardell et al. 1980a). From chemical pulps, vessels can be separated either by sieving (Fig. 2.4) or by a special technique involving defibration using only a jet of water and avoiding mechanical action. The pulp is then allowed to dry slowly at room temperature, after which it is resuspended in water with a mixer. When the slurry is allowed to settle, the vessels will float and can be skimmed off (Marton and Agarwal 1965).

2.4 Analysis of Cell Wall Layers and Middle Lamella

As already mentioned, several methods are available for the direct determination of the distribution of lignin across the cell wall and in the middle lamella (Chap. 1). These methods include ultraviolet (UV) microscopy and scanning and transmission electron microscopy (SEM and TEM) coupled with energy-dispersive X-ray analysis (EDXA or EDS). A special technique involving the combination of UV microscopy with EDXA has been applied for studying the variations of the guaiacyl-to-syringyl lignin ratio in the walls of various hardwood cells (Saka et al. 1982).

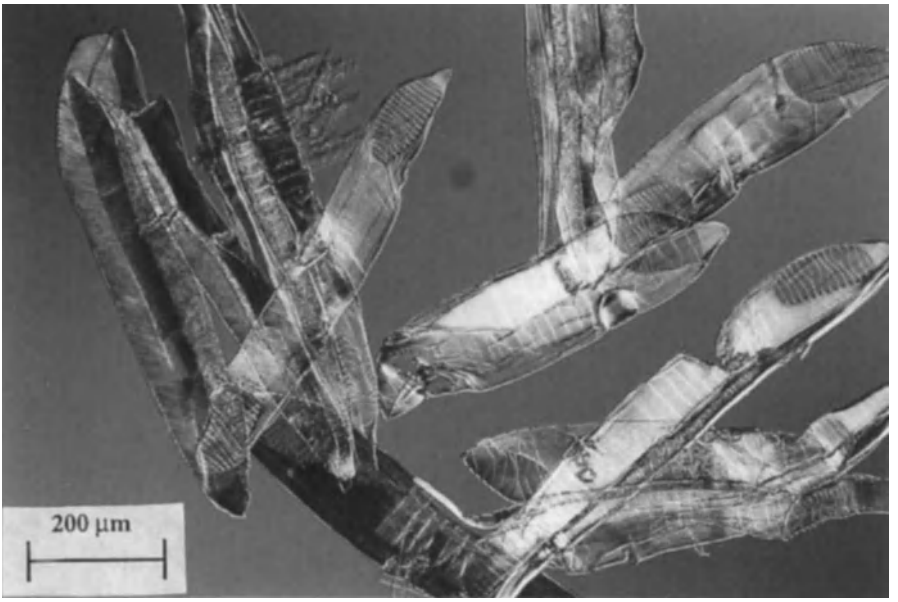

Fig. 2.4. Vessels isolated from a birch (*Betula verrucosa*) kraft pulp

Without a preceding isolation of material from the respective morphological regions, it is not possible to study the distribution of carbohydrates in the cell wall layers. Also, for more extensive studies on the differences in the structure and reactivity of lignin, its selective isolation from the cell wall layers and middle lamella is necessary.

Because of experimental difficulties, entailing tedious and special techniques, it is not surprising that conflicting results have been reported in the literature, especially concerning the variations in lignin structure across the cell wall. More work is still required for improving both the analytical and the preparative methods.

2.4.1 Direct Determination of Lignin

For quantitative assay of lignin distribution by UV microscopy, thin sections (0.1–0.5 µm) are needed, which are prepared from embedded wood material by using a microtome (Scott et al. 1969). The measurements are made around the absorption maximum of lignin at 280 nm. However, the lignin concentration cannot be measured precisely, because it is not known how the absorptivity values vary across the cell wall. In the case of hardwoods, the situation is especially complicated because the proportion of guaiacyl and syringyl

residues, having specific contributions to the absorptivity, is different in different types of cells and probably also variable (Fergus and Goring 1970; Musha and Goring 1975).

The SEM-EDXA and TEM-EDXA measurements are also performed using embedded and thin wood sections, but the lignin must first be labelled with atoms (e.g., bromine or mercury) detectable by EDXA. A prerequisite for the quantitative analysis is a uniform and specific labelling. Bromination is an electrophilic substitution reaction of the aromatic ring and it is highly dependent on the reaction conditions. A homogeneous mono- or dibromination of the ring is a prerequisite for quantitative results, but difficult to obtain because the reactivity varies across the cell wall. However, for semiquantitative measurements, the bromine labelling (at room temperature) is a convenient technique. Samples of wood sticks are treated in pyridine in the presence of bromine (Saka et al. 1982, 1988). After bromination, the sample is thoroughly washed with solvent to remove the residues of bromium salts, formed during bromination. TEM-EDXA for which ultrathin sections are used, results in a better resolution than SEM-EDXA.

EDXA has recently been improved by substituting mercurization for the nonspecific bromination step (Eriksson et al. 1988; Westermark et al. 1988). In mercurization, the reactivity of lignin is lower compared with bromination, resulting in the introduction of only one mole of mercury per aromatic ring. The same ratio is obtained for softwood, hardwood, and reaction wood lignin. Mercurization is performed by treating small wood sticks with mercuric(II) acetate in methanol at 90 °C for several hours. The mercurized wood samples are then embedded and sectioned by standard techniques before analysis.

2.4.2 Wood Material for Separation Studies

In order to isolate material from different morphological regions, wood must first be disintegrated into very small particles. Only carefully selected material should be used, avoiding contamination of compression wood with its exceptionally high content of lignin with a differing structure (guaiacyl-*p*-hydroxyphenyl lignin). Various grinders (British standard disintegrator, conical refiners, ball mills, air-jet disintegrator, etc.), have been used (Sorvari et al. 1983). The material obtained (after grinding under wet or dry conditions) is then divided into separate fractions by using either the density gradient separation technique (Sect. 2.4.3) or mechanical fractionation (Sect. 2.4.4).

Instead of disintegrated wood, thermomechanical pulps (TMP) have been used as starting materials for the separation of constituents from different morphological regions, because these pulps contain different wood and fiber fragments. The wood polymers can, however, be at least slightly depolymer-

ized and partially damaged during the mechanical treatments, a possibility that deserves careful consideration. The mechanical treatment of wood should therefore be as mild as possible and higher temperatures should be avoided. In the case of TMP, fractions should be used as a starting material after a relatively short refining time.

2.4.3 Density Gradient Separation of Middle Lamella and Secondary Wall Material

Fine particles obtained after disintegration of extracted wood (fraction passing a 500-mesh or a 25-μm sieve) are used for the fractionation of lignin-rich material (mainly the middle lamella including cell corners) from carbohydrate-rich fiber material (mainly the secondary wall). The separation method is based on the different densities of lignin (≈1.33) and polysaccharides (1.55–1.59, depending on the degree of crystallinity). In a tube filled with a liquid of proper density, the material is separated into two layers according to their densities (lignin-rich material in the upper and polysaccharide-rich material in the lower layer). By using this technique in combination with proper grinding methods, it has been possible to obtain from softwood two fractions of differing lignin contents, namely, a middle lamella fraction with a maximum lignin content of about 50% and a secondary wall fraction with a minimum lignin content of 20% (Whiting et al. 1981; Sorvari et al. 1983, 1986).

The separation liquid is prepared by mixing a heavy solvent, usually carbon tetrachloride with light solvents, such as 1,4-dioxane, *p*-xylene, or hexane. The density of the solvent mixture is determined accurately (to at least three decimals) by a pycnometer or gravimetrically. The slurry of wood powder in the solvent mixture should be carefully deaerated by ultrasound. Because of the tendency for formation of aggregates, it is advisable to use centrifugation (10 000 rpm for 15 min) and conical flasks for an easy recovery of the fractions (Whiting et al. 1981). Their purity can be checked by lignin analysis and by microscopy, preferably using polarized light. The middle lamella and primary wall lack birefringence in contrast to the fractions containing material from the secondary wall.

2.4.4 Mechanical Separation of Middle Lamella, Primary Wall Material, and Ray Cells

The mechanical separation is based on the differences in the size and shape of cells and cell fragments. Disintegrated wood or TMP pulps slurried in water are suitable starting materials. The fractionation is performed using

wet screening with a set of sieves and a small laboratory hydrocyclone. The fractionation is followed by stereomicroscopy (magnification 100–200) and the purity of fractions is controlled both by chemical analyses and microscopy.

The method (Hardell et al. 1980a,b; Westermark 1985) is illustrated in Fig. 2.5. The fibers retained on a 50-μm sieve are first removed from the

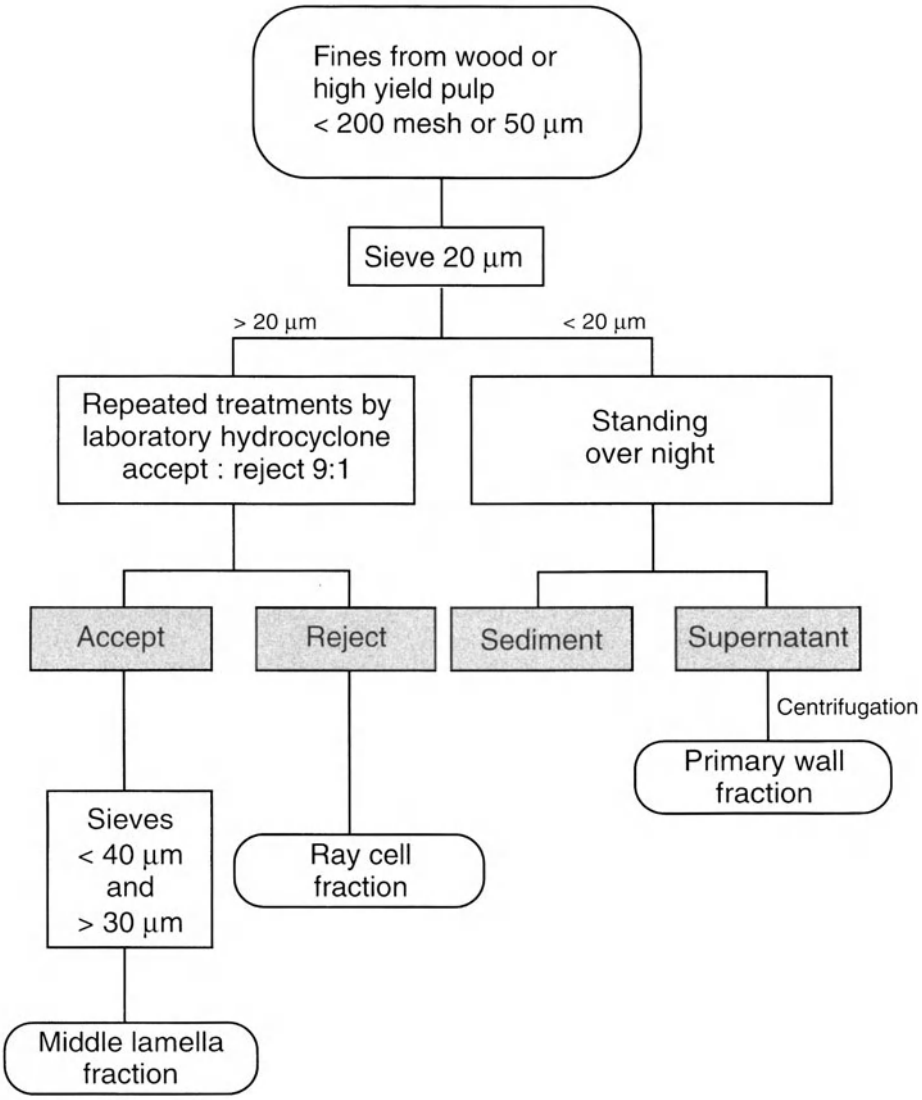

Fig. 2.5. Fractionation scheme for isolation of middle lamella fragments, primary wall material, and ray cells

starting material. The material from both middle lamella and ray cells is collected in the fines passing a 50-μm but retained on a 20-μm sieve. Their separation is effected by using the hydrocyclone (accept-to-reject flow ratio ≈9:1) and the ray cells are collected from the reject. The procedure is repeated several times until a pure ray cell fraction is obtained. The middle lamella particles are collected from the accept and their final purification is effected by sieving. The fraction passing the 40-μm but retained on the 30-μm sieve is collected. An acceptable fraction of material from the middle lamella has a high lignin content (preferably at least 40%) and it appears under the microscope as flaky particles with typical cell corners (Fig. 2.6). This fraction should show no or only a weak birefringence when examined in polarized light.

The particles passing the 20-μm sieve are used for the separation of primary cell wall material. The slurry is allowed to settle overnight after which the fine particles from the supernatant liquid are collected by centrifugation. The enriched primary wall material should have a lignin content of around 40% and shows a low birefringence in polarized light. This fraction typically contains some nitrogen (2–3%), and it appears in the scanning electron microscope as very thin threads (Fig. 2.7). To avoid bacterial contamination of this fine fraction, only fresh starting materials should be fractionated at relatively low temperatures.

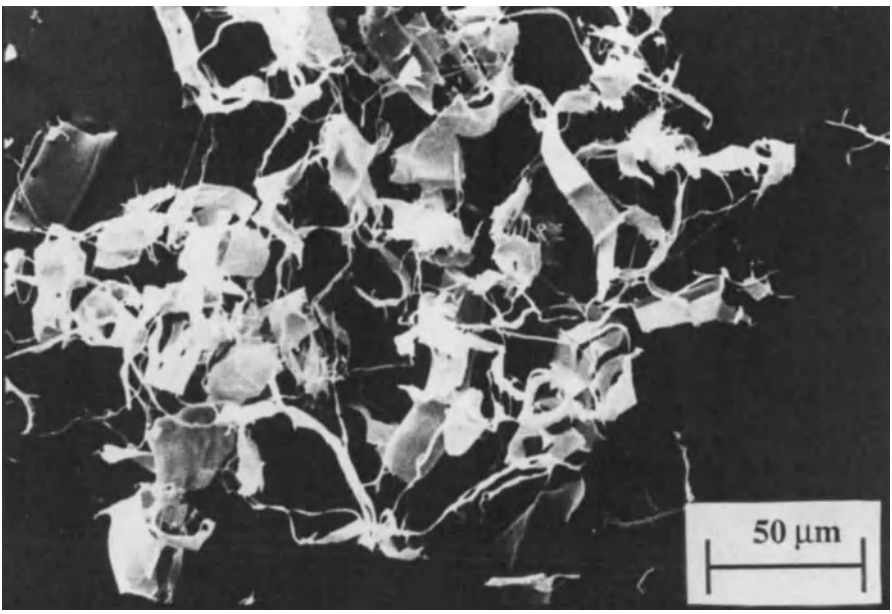

Fig. 2.6. Middle lamella fragments isolated from Norway spruce (*Picea abies*) by fractionation. Scanning electron micrograph

2.4.5 Isolation of Middle Lamella from Soft Xylem

The method for isolating the middle lamella from soft xylem can sometimes be useful although it is a tedious procedure. Soft xylem is formed from the cambial cells at the beginning of the growth period. The method is based on the observation that lignification lags behind the synthesis of the carbohydrate matrix and it begins at the cell corners in the middle lamella. The lignin in soft xylem is exclusively located in the middle lamella-primary wall region (M + P). Soft xylem is collected from the cambial zone early in the spring, two to three weeks after the initiation of annual growth (Meshitsuka and Nakano 1985). Removal of the bark causes a rupture of the cambial zone with the true cambium at the bark side and the newly formed cells at the xylem (wood) side. These new cells can be removed from the wood by gentle scraping using a soft, blunt piece of plastic. The samples isolated should have a lignin content of less than 10–15%. When the method is performed properly and progress of lignification is followed under the microscope, it can be a valuable complement in the studies of middle lamella lignin. However, the middle lamella isolated in this way is only partially lignified and the structure and properties of this lignin can deviate from that present in the middle lamella of fully developed tissues. Material from lignin-free primary walls can be isolated from the cambial zone by very gentle scraping of the bark side.

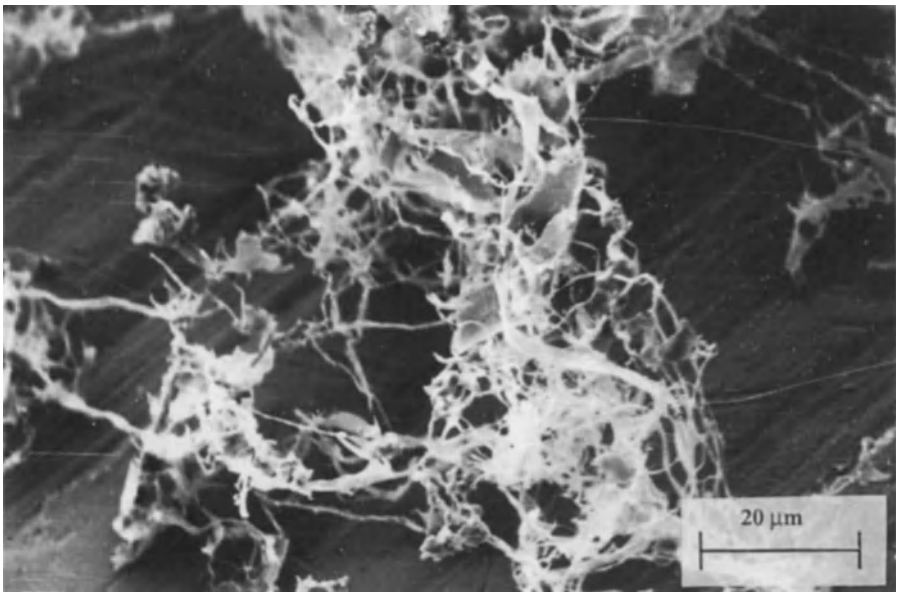

Fig. 2.7. Primary wall fraction isolated from Norway spruce (*Picea abies*). Scanning electron micrograph

2.4.6 Isolation of Cell Wall Layers from Differentiating Xylem

Two techniques for isolation of the cell wall layers with the aid of a micromanipulator have been described. In the middle of the 1930s, A. J. Bailey (1936) succeeded in isolating 0.5 mg of material from the middle lamella of Douglas fir wood. His tedious manual procedure for which he used a special tool, has not yet been repeated. Another technique was developed for the analysis of the distribution of polysaccharides in the cell walls in the beginning of the 1960s (Meier and Wilkie 1959; Meier 1961) but this technique is also tedious and it has not been applied lately. Fibers at different stages of maturation were isolated by radial sectioning of wood through the outermost part of the xylem. The final separations were made using a micromanipulator under a polarizing microscope and tracheids and fibers were divided into four fractions representing various stages of growth; namely M + P, M + P + S_1, M + P + S_1 + S_2 outer, and M + P + S_1 + S_2 outer + S_2 inner.

2.4.7 Peeling of Cell Wall Layers from Pulp Fibers

The outer layers of chemical pulp fibers can be separated by using a special peeling technique. The British disintegrator is especially suitable for this purpose because the treatment is mild without a cutting action on the fibers. The fine fraction is first removed from the pulp by sieving and the fibers are then disintegrated at about 1% consistency for different lengths of time (a variation of the method is to use ethanol:water 1:1 at 3% consistency). By this technique it is possible to remove gradually the cell wall layers beginning with P and S_1 and then S_2 (Kallmes 1960; Purina et al. 1994). The purity of the cell wall layers is, however, difficult to control. Even if the material from the primary wall can be recognized from its lack of birefringence, it is difficult to distinguish the material from the different layers of the secondary wall. Scanning electron microscopy of the peeled fibers can give an idea of how the peeling proceeds.

A very mild treatment of the fibers at a higher consistency (4.5%) can be used for removal of only residues from the middle lamella and primary wall of the fiber surface (Heijnesson et al. 1995).

Finally, it should be mentioned that another, and a quite different type of technique compared with the previous one, has been suggested, according to which the pulp fibers are first acetylated under nonswelling conditions so that only the periphery of the fibers reacts. The outer fiber layer containing acetylated polysaccharides is then peeled by extraction with an organic solvent and, finally, the resulting solution as well as the peeled fibers are analyzed (Luce 1964). By repeated treatments (acetylation plus peeling), information can be obtained about both the distribution of hemicelluloses and the DP of cellulose

across the fiber cell walls. However, since the appearance of the original article, no studies confirming the usefulness of this interesting technique have been published.

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3 Carbohydrates

T. Vuorinen and R. Alén

3.1 Introduction

The major wood polymers are packed in a highly ordered pattern during biogenesis of the cell wall (Terashima 1990, 1993). Cellulose microfibrils, each formed of about 100 cellulose chains, are embedded in a matrix of hemicelluloses and lignin. Pores in the matrix are small and enable transfer of water, salts, and small organic molecules only. Although the hemicelluloses are at least partly water soluble, they cannot diffuse out of the cell wall due to their molecular size.

Complete identification of polysaccharides requires their isolation in a pure state. Because of their characteristic arrangement in the cell wall, a specific strategy is needed to extract hemicelluloses from wood, but otherwise their purification and chemical analysis is performed with methods that are commonly applied in carbohydrate chemistry.

The basic structures of cellulose and the major cell wall polysaccharides are well known and they are therefore generally quantified by analysis of the monosaccharide composition of whole wood or pulp material. The isolation of pure cell wall polysaccharides begins with preparation of essentially extractive- and lignin-free holocellulose. The removal of lignin is necessary because it makes the cell wall porous and enables the hemicelluloses to diffuse out through the intercellular spaces. Fractional extraction of hemicelluloses can be based on their solubilities in neutral and alkaline solvents.

Traditionally, impure hemicellulose fractions have been purified by fractional precipitation. Today, different modes of column chromatography offer a more sophisticated and controllable alternative.

Chemical characterization of pure polysaccharides proceeds in steps starting from determination of monosaccharide composition and going through assignment of linkage positions to full sequence analysis. Anomeric configuration and molecular weight are obtained with separate methods. Both theoretical (computational) and experimental techniques are available for evaluation of the three-dimensional structure of polysaccharides. The treatise in this chapter follows the same order of increasing complexity with the exception that methods for analysis of three-dimensional structures are not covered.

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3.2 Isolation and Fractionation of Polysaccharides

In wood-based samples, cellulose is present in close association with other cell wall polysaccharides (hemicelluloses) and lignin (Terashima 1990, 1993). The main scheme for fractionation of wood polysaccharides involves preparation of extractive- and lignin-free holocellulose followed by fractional extraction of hemicelluloses (glucomannans and xylans) (Browning 1967; Aspinall 1982a; Birch 1985; El Khadem 1988; White and Kennedy 1988a; Fengel and Wegener 1989; Easty and Thompson 1991; Morohoshi 1991; Sjöström 1993). Some wood species contain large amounts of extracellular polysaccharides, such as arabinogalactan in larch wood, which can be extracted directly from undelignified wood. Mechanical disintegration of wood can also make the cell wall polysaccharides partially accessible for solvents without preparation of holocellulose.

3.2.1 Preparation of Holocellulose

To enable extraction of hemicelluloses from the cell wall, its porosity must be increased. This is brought about by selective removal of lignin from extractive-free wood meal. The residue, consisting of cellulose and hemicelluloses, is called holocellulose (Fig. 3.1).

Selective delignification of wood meal is possible only with methods that degrade lignin into fragments small enough to pass through the native, small pores of the cell wall. All common methods apply electrophiles, or form them in situ, because these can oxidize both phenolic and nonphenolic lignin structures. Reagents most commonly used include an acid solution of sodium chlorite or chlorine gas. In addition, a mild delignification can be obtained by the use of dilute peracetic acid.

Ideally the selected delignification procedure should result in a total removal of lignin without dissolution and chemical modification of cellulose and hemicelluloses. However, there is no method able to completely satisfy this requirement. The reactions of delignifying agents with the wood polysaccharides can be divided into oxidative conversion of functional groups and hydrolytic degradation of the polysaccharide chains. In general, some reducing aldehydic end groups (hemiacetal structures) as well as primary and secondary hydroxyl groups are oxidized, resulting in the formation of carboxyl and carbonyl groups. Some deacetylation of hemicelluloses can also occur. In practice, preparation of a holocellulose always involves some loss of carbohydrates ($<5\%$ of the total amount) and, in most cases, also retention of small amounts of lignin (residual lignin).

The standard acidic chlorite procedure entails the treatment of extractive-free wood with an aqueous solution of sodium chlorite at pH 4 at 70–80 °C for

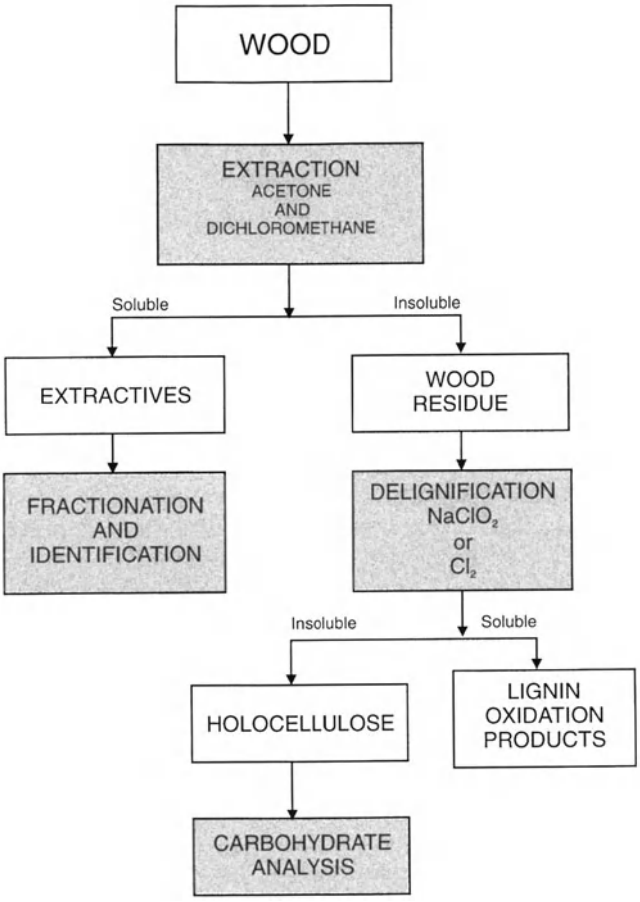

Fig. 3.1. Preparation of holocellulose

3–5 h. Although chlorite itself is quite unreactive, more aggressive chlorine, hypochlorous acid, and chlorine dioxide are formed in situ. The chlorination method includes delignification with chlorine gas at 0 °C for 5 min followed by extraction with hot 2-aminoethanol in ethanol. Chlorination and washing stages are repeated until the sample is white or does not become lighter upon further chlorination.

The acidified sodium chlorite method is usually applied to both softwoods and hardwoods, whereas the milder chlorination method is especially useful for the study of hardwoods. Because the chlorination method applies non-aqueous solvents only, even the most hydrophilic hemicelluloses are retained, unlike in the chlorite method. On the other hand, the extraction of oxidized lignin with hot 2-aminoethanol/ethanol can lead to partial deacetylation of hemicelluloses. Oxidation of reducing end groups of polysaccharides is more extensive under harsher conditions of the chlorite method.

In addition to standard chlorine and chlorite methods, several modifications have been proposed to further minimize the degradation of the carbohydrates. The modified methods often combine lower temperatures with prolonged reaction times. Toward the end of delignification all procedures entail losses of polysaccharides. One drawback in the preparation of holocellulose is that possible chemical linkages between polysaccharides and lignin are destroyed.

3.2.2 Fractional Extraction of Hemicelluloses and Pectins

Hemicelluloses can be isolated from wood, holocellulose, or pulp by extraction with an aqueous alkali (KOH or NaOH). To minimize degradation of the reducing end groups by alkali, sodium (or potassium) borohydride can be added, but the cleavage of acetyl groups is a disadvantage that cannot be avoided. Among the few neutral (nondestructing) solvents, dimethyl sulfoxide (DMSO) is useful, particularly with respect to xylans even if the extraction yield is relatively low (<50%). Less well known but powerful solvents dissolving even cellulose are *N*-methylmorpholine *N*-oxide and lithium chloride/dimethylformamide, but they have not been used for fractionation of wood polysaccharides at a preparative scale.

Sodium hydroxide is a better solvent for glucomannans than potassium hydroxide, but the latter is sometimes preferred because its neutralization product with acetic acid (potassium acetate) has a higher solubility than sodium acetate in ethanol. Addition of boric acid (borates) to the alkaline extraction solution facilitates the dissolution of (galacto)glucomannans because the borate ions form anionic addition products (with the vicinal hydroxyl groups of mannose units at C-2 and C-3 positions), which have a higher solubility in an alkali (Fig. 3.2). The adducts are readily decomposed on acidification.

A gradient extraction with varying alkali concentrations can be used for a rough fractionation of hemicellulose components (Figs. 3.3 and 3.4). For example, the sample (holocellulose or pulp) is first extracted with DMSO and then successively with alkali (NaOH or KOH) with increasing concentrations. The final elution is made with a relatively strong alkali in the presence of borate. To precipitate the hemicelluloses, each fraction is neutralized by adding acetic acid. Addition of a neutral organic solvent, usually ethanol, to the neutralized (and filtered) fractions results in further precipitation of hemicelluloses. Some more specific precipitation agents are also known, for example, barium hydroxide for glucomannan (barium ions form complexes with 2,3-vicinal hydroxyl groups on mannose units) and cetyltrimethylammonium hydroxide for glucuronoxylan (Fig. 3.2).

After a complete extraction, cellulose remains as the solid residue but it still contains traces of hemicelluloses that cannot be removed completely. During

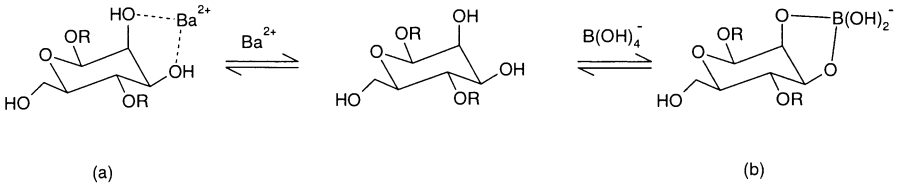

Fig. 3.2. Reaction of the *cis*-2,3-diol structure of a 1,4-linked β-D-mannopyranosyl unit with **a** barium and **b** borate ions

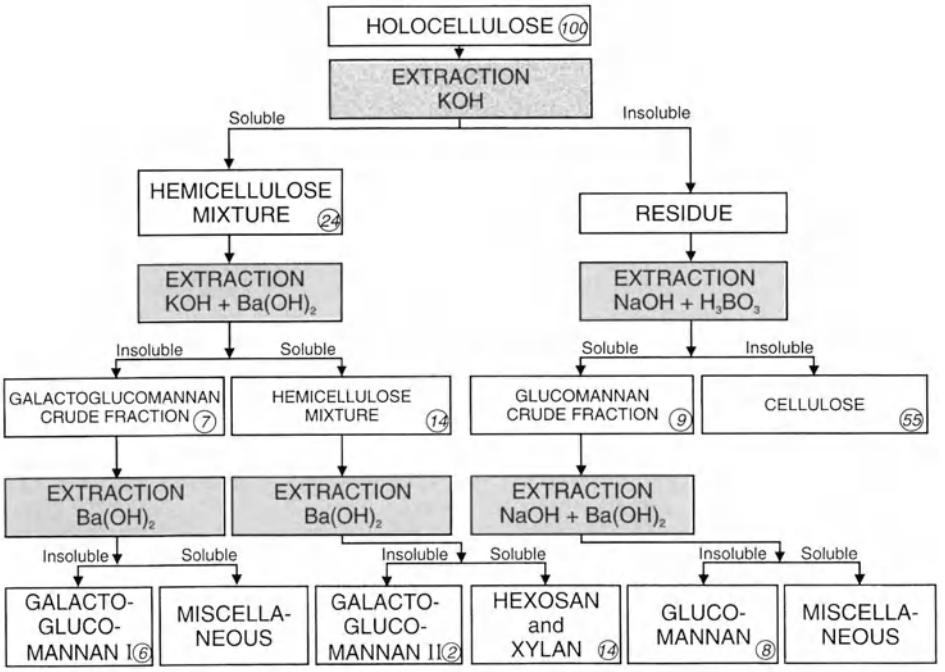

Fig. 3.3. Isolation of galactoglucomannans from softwood holocellulose by alkaline extractions with sodium hydroxide and sodium borate. In all cases, the soluble fractions of hemicelluloses are precipitated with acidified ethanol. Data in *circles* denote material balance in percentage (Timell 1961)

extractions, cellulose is at least to some extent degraded and depolymerized. A possibility for a selective isolation of a pure undegraded cellulose is a direct nitration of wood and extraction of the resulting cellulose trinitrate by organic solvents (Chap. 1).

TAPPI standards T 203 om-93 and 429 cm-84 and CPPA technical section standard G.29P describe a procedure for isolating "pure" cellulose (α-cellulose) through successive extractions with 17.5% and 9.45% sodium hydroxide at 25 °C. Even if the α-cellulose is not completely pure and undegraded, it

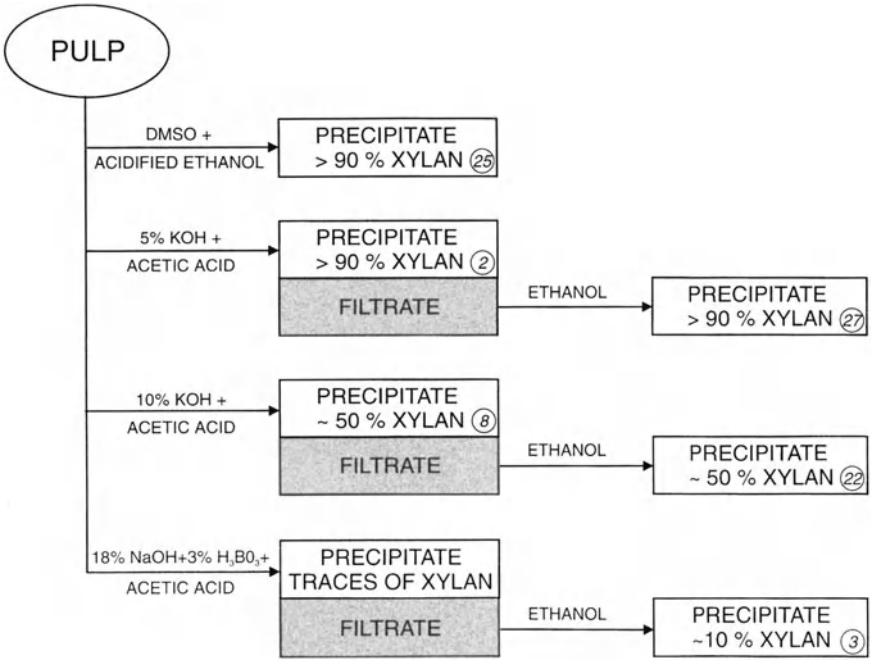

Fig. 3.4. Isolation and fractionation of xylan from bleached kraft pine pulp with successive extractions with dimethyl sulfoxide (DMSO) and alkali. The data as *percentage* refer to the purity of xylan in the fractions and the data in *circles* indicate the extraction yield of xylan (percent of the xylan in pulp). Note that xylan can be extracted with a rather high selectivity with DMSO and 5% KOH, but increasing amounts of other polysaccharides (mainly glucomannan) are present in the next fractions. Adopted from Sjöström and Enström (1967)

represents the higher molecular weight cellulose fraction of the sample. The β-cellulose (precipitable from the alkaline extracts on neutralization) is defined as a degraded cellulose while the γ-cellulose (soluble fraction after neutralization) refers mainly to hemicellulose residues. These methods, including the terms α-cellulose, β-cellulose, and γ-cellulose, date from old times and at least in the case of paper-grade pulps it is doubtful whether they are of any practical value.

In some cases, and in order to avoid degradation during preparation of holocellulose, wood meal can be directly extracted with alkali. A rather good yield of xylan is obtained for certain hardwood species, such as poplars, but the extract must be freed from lignin and other impurities afterward. In the case of softwoods, preparation of holocellulose is necessary before extraction of hemicelluloses. An exception is, of course, arabinogalactan in larches, which is readily extractable with water.

Pectins and/or pectic acids are located in the M + P layers. It is generally believed that they exist in the middle lamella as calcium salts and in the

primary wall as methyl esters. Aqueous solutions of chelating agents, e.g., sodium hexametaphosphate, decompose the tightly coordinated gels and enable extraction of pectins.

3.2.3 Purification of Hemicelluloses

A rough fractionation of the hemicelluloses can be achieved by successive extractions with neutral or alkaline solvents. However, the separated preparations are in all cases still mixtures of different hemicelluloses and, especially for detailed structural analyses, must be further purified to obtain homogeneous fractions. In many cases, it may also be necessary to obtain pure hemicellulose fractions from pulping and bleaching liquors. The purification techniques available include fractional precipitation, enzymic hydrolysis, and column chromatography.

In general, glucomannans are separated from associated xylans by precipitation with barium hydroxide. A selective and almost quantitative precipitation is possible. This is due to the fact that barium hydroxide forms insoluble complexes with glucomannans based on the reaction of barium hydroxide with the *cis*-2,3-diols of mannopyranose units. Other precipitating agents for glucomannans are Fehling's solution or other copper salts as well as basic lead acetate. In particular, acidic polysaccharides such as glucuronoxylans form precipitable complexes with cetyltrimethylammonium bromide or hydroxide. In addition, borate complexes allow selective salt formation with cetyltrimethylammonium hydroxide to be used, for example, in the fractionation of neutral arabinogalactans.

Dissolved hemicelluloses have often been recovered from alkaline extracts by acidification (mainly with acetic acid) and subsequent additions of increasing amounts of ethanol, acetone, or another neutral organic solvent. A good fractionation requires several precipitation steps and is therefore more tedious than many other approaches. The solubility differences between hemicelluloses can also be too small to allow a complete purification.

Among the common organic solvents 1,4-dioxane and methyl cellosolve are the best solvents for lignin and therefore especially suitable for a selective precipitation of hemicelluloses from lignin-rich solutions such as kraft cooking liquors (Fig. 3.5). Co-precipitation of free lignin is minimal, whereas some lignin linked chemically to hemicelluloses is recovered. Not all hemicelluloses are, however, precipitated, as they vary much in their solubility. Thus, for example, kraft cooking liquors contain polysaccharides rich in arabinose that are exceptionally soluble and are mostly retained in the liquid phase after precipitation with 1,4-dioxane/acetic acid (Engström et al. 1995).

Various precipitation techniques have also been applied for purification of hemicelluloses from inorganic or organic salts. Desalting through dialysis or ultrafiltration is, however, more quantitative and convenient. When coupled

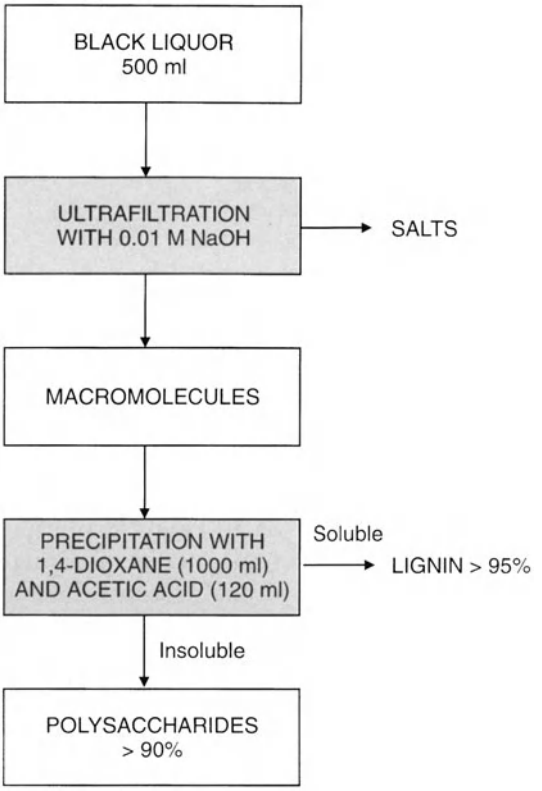

Fig. 3.5. Isolation of hemicelluloses from kraft cooking liquor by precipitation with 1,4-dioxane/acetic acid

with freeze-drying, clean, powdered samples with minimal chemical changes can be prepared.

Impure hemicellulose preparations can be further fractionated and purified by gel permeation chromatography (GPC) and anion exchange chromatography (AEC; York et al. 1985). GPC fractionates hemicelluloses according to their molecular weight. The technique is useful for the separation of hemicellulose mixtures containing variable lignin residues. In principle, GPC could also be applied for desalting, but precipitation of both xylans and glucomannans from pure water on the column material may prevent this approach.

For preparative purposes, AEC on diethylaminoethyl (DEAE) anion exchanger-based columns in the acetate or formate form is generally used for the purification of polysaccharides containing carboxylic acid groups. Elution with solutions with increasing salt content (sodium acetate or formate) and alkali (sodium hydroxide) concentration gradually force even the most acidic components through the column (Fig. 3.6). AEC can be extended to the sepa-

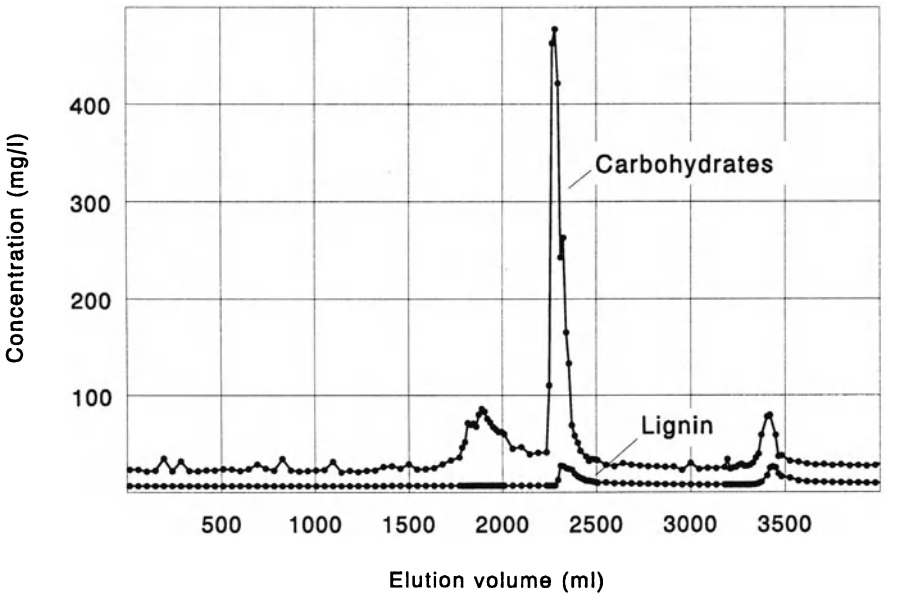

Fig. 3.6. Fractionation of hemicelluloses from kraft cooking liquor by AEC. Carbohydrate and lignin contents were monitored by phenol/sulfuric acid assay and UV spectrophotometry, respectively. (Engström et al. 1995)

ration of neutral polysaccharides capable of forming charged complexes, e.g., with borate ions. In this application, polysaccharides forming borate complexes are retained on columns in the borate form and can be eluted with borate solutions of increasing strength.

In comparison with the precipitation methods, AEC is more likely to lead to clean hemicellulose fractions. In some cases, AEC may indicate the presence of complex structures that could not be fully identified by precipitation techniques only (Engström et al. 1995). For example, strong evidence on hemicellulose-hemicellulose and hemicellulose-lignin complexes was obtained through fractionation of polysaccharides from kraft cooking liquors by precipitation followed by AEC and GPC (Fig. 3.6).

Specific enzymes can also be used for purification of hemicellulose mixtures. Depolymerization of the contaminating glycan backbones with endoglycanases, followed by GPC offers a convenient way, for example, to purify mixtures of galactoglucomannans and xylans. However, enzymes need to be applied with care to unknown samples because any information on rare structures and bonding between polysaccharide chains is easily lost (Tamminen et al. 1995).

3.3 Detection of Carbohydrates

It is often useful to obtain a preliminary and rapid estimation of the quantity and type of soluble carbohydrates present in the isolated preparations or monitor selectively the elution of carbohydrates in column chromatography. In general, the detection methods are based on formation of furfural and 5-hydroxymethylfurfural and their condensation with a color reagent, oxidation of 1,2-diol structures, or reduction of colored metal ion complexes by free aldoses and ketoses.

At high temperatures, strong acids dehydrate pentosans and hexosans to furfural and 5-hydroxymethyfurfural, respectively. Both products strongly absorb UV light at about 280 nm. If the reaction is carried out under controlled conditions, the absorbance is proportional to the total carbohydrate concentration of the sample. In wood-derived samples, background absorption may be caused by lignin. If the dehydration is carried out in the presence of certain color reagents, they are condensed with furfural and 5-hydroxymethylfurfural, thus shifting the absorption to the visible region of light. This usually leads to less interference from noncarbohydrate components and often to an increased sensitivity. The color reagents most commonly applied include phenol, anthrone, and orcinol, resulting in absorption maxima at 490 (yellow), 620 (green), and 665 nm (blue), respectively.

The tests based on dehydration of carbohydrates are especially suitable for polysaccharides because the reactions occur almost instantaneously in one treatment. Another general reaction of carbohydrates is their oxidation by periodate. The oxidation varies depending on the configuration of free 1,2-diols. Thus, *cis*-1,2-diols begin to react at room temperature, whereas *trans*-1,2-diols require heating at higher temperatures (Fig. 3.7). Because both structures consume equal amounts of periodate, its consumption is related to the total carbohydrate concentration of the sample. The concentration of periodate is conveniently followed by its strong UV absorption at 220 nm. One advantage of the periodate oxidation is that it can be performed under neutral conditions that are suitable for most commercial post-detectors or flow analyzers (Nordin 1983).

Several rapid tests are available for determination of reducing sugars in solution (York et al. 1985). These are not generally as useful for the detection of polysaccharides as the tests mentioned above because the hydrolysis of glycosidic bonds and the test itself must be applied in two separate steps. This is, however, not a problem for flow analyzers that can easily handle several subsequent reaction steps. All glycosidic bonds are hydrolyzed with dilute mineral acids at higher temperatures, whereas enzymic hydrolysis liberates reducing sugars selectively from only certain linkage types. Indeed, the hydrolysis of polysaccharides by enzymes is routinely followed by tests that detect reducing sugars (e.g., the 3,5-dinitrosalicylic acid method). At present, a great number of standardized enzymic tests are available for quantification of individual

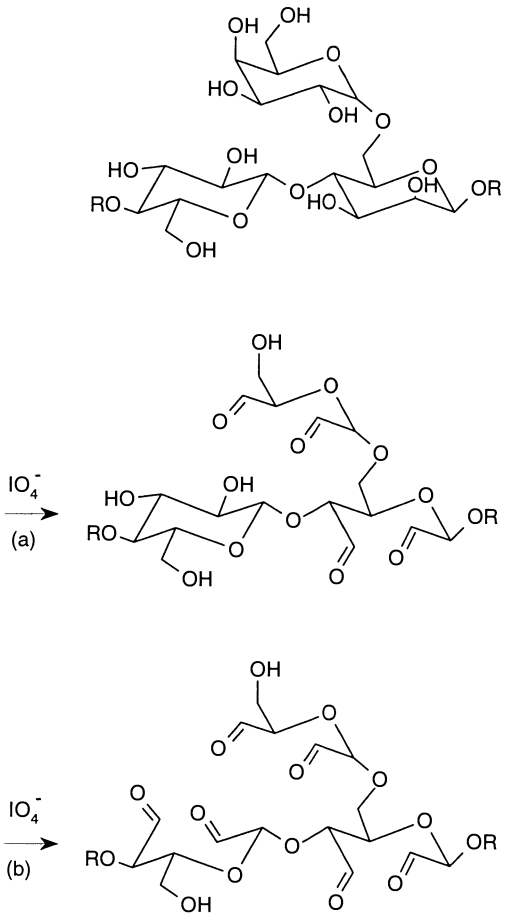

Fig. 3.7. A schematic illustration of the oxidation of galactoglucomannan with periodate showing **a** fast reaction of galacto- and mannopyranosyl units and **b** complete oxidation under extended conditions

monosaccharides, based on their dehydrogenation or other reactions (White and Kennedy 1988b).

Quantification of reducing sugars is most commonly based on their oxidation by certain metal ions. Changes in the oxidation level of the metal ion are followed in the presence of a complexing agent that changes the color of the solution depending on the valence of the metal ion. Generally, the reducing sugars are oxidized by cupric ions with formation of cuprous ions. In the traditional Fehling's reaction, oxidation of cupric sulfate to cuprous oxide is followed. Another more recent test utilizes neocuproine as a complexing agent.

Reducing sugars also react with alkaline ferricyanide (above pH 10.5) on heating, forming a ferrocyanide derivative that reacts with ferric salts to produce Prussian blue. In the presence of reducing sugars, the Nelson-Somogyi method (using an arsenomolybdate/copper reagent) and the Molisch test

(using 2-naphthol in ethanol or in chloroform in the presence of sulfuric acid) produce a blue or a dark purple solution, respectively. With Schiff's reagent (*p*-rosaniline hydrochloride in a cold, freshly prepared, saturated aqueous solution of sulfur dioxide) aldehydes produce a pink color, while ketones are without effect.

3.4 Monosaccharide Composition

Determination of the monosaccharide units in polysaccharide samples involves cleavage of glycosidic bonds and a subsequent analysis of the monomers, most commonly, by a chromatographic technique. The determination is normally made either to measure the total content of cellulose and hemicelluloses in a wood or pulp sample, to elucidate the chemical composition of a pure sample of the isolated polysaccharide, or to quantify dissolved polysaccharides in pulping, bleaching, and papermaking processes. Calculation of polysaccharide composition of wood and pulps is generally based on several assumptions on the structure of the major hemicelluloses (Janson 1970). Although all details are not included, the calculations give reasonably accurate values for the contents of cellulose, (galacto)glucomannans, and (arabino)glucuronoxylans.

Depending on the sample, glycosidic bonds are cleaved by hydrolysis or methanolysis (Wayman 1986; Fan et al. 1987; Biermann 1989b; De Ruiter et al. 1992; Henrissat 1994). Cellulose-containing samples are hydrolyzed with strong mineral acids or trifluoroacetic acid, which are able to destroy the crystalline structure of cellulose. In comparision with acid hydrolysis, methanolysis is fast but can only be applied for quantification of noncrystalline samples. These samples can also be hydrolyzed with enzyme mixtures (Buchert et al. 1993). Each of the techniques has limitations and advantages that will be further discussed below.

The liberated monosaccharides, or their derivatives, are usually separated and quantified by gas chromatography (GC), high-preformance liquid chromatography (HPLC), or AEC. More recently, separation of monosaccharides with capillary electrophoresis (CE) has become possible. The modern techniques and instrumental methods of analysis permit carbohydrate determination at very low concentrations making it, for example, possible to characterize the polysaccharides even from different parts of the cell wall of a single fiber.

3.4.1 Cleavage of Glycosidic Bonds

Independent of the method applied, the conditions for the cleavage of glycosidic bonds must be selected so that complete hydrolysis or methanolysis is

achieved with little or no degradation of the monosaccharide units. In practice, due to the different sample matrices and stabilities of different glycosidic linkages between varying monosaccharide units, the use of more than one set of hydrolysis conditions may be necessary. Most notably, complete hydrolysis of cellulose requires conditions under which its crystalline structure swells, making it accessible to the acid.

Sulfuric acid is most commonly used for hydrolysis of polysaccharides. In the usual method, complete hydrolysis of cellulose samples is accomplished in two steps (cf. TAPPI standard T 249 cm-85). Impregnation, swelling, and preliminary hydrolysis involve treatment of the sample with 72% sulfuric acid for 1 h at 30 °C. The final hydrolysis requires dilution of the sulfuric acid to 4% and refluxing for 4 h or heating for 1 h in an autoclave at 120 °C. After hydrolysis, the acid must be neutralized, preferably with an anion exchange resin, prior to derivatization and/or chromatographic analysis.

In order to minimize the nonspecific degradation during hydrolysis with sulfuric acid, a number of alternative methods have been proposed, including the use of trifluoroacetic acid (TFA). Water- or alkali-soluble polysaccharides are normally refluxed in 2 M TFA for 1 h. Cellulose containing samples are steeped in concentrated TFA for 15 min and then successively refluxed for 15-min periods in concentrated and 80% TFA. A final 30-min reflux in 30% TFA completes the hydrolysis of cellulose. After repeated concentration on a vacuum rotary evaporator, with the addition of water after the first concentration, the sample is ready for chromatographic analysis. The TFA hydrolysis is rapid for noncrystalline samples due to the simple deacidification through evaporation, whereas for crystalline samples this advantage is reduced by the number of hydrolysis steps.

Difficulty is experienced in achieving a quantitative determination of the 4-*O*-methyl-D-glucuronic acid units in hemicellulose hydrolyzates, because the glycosidic bond between the uronic acid and monosaccharide units in the xylan is more resistant to acid hydrolysis than those between the neutral monosaccharide units. Conditions necessary for complete cleavage of this glycosidic bond with sulfuric acid or TFA cause severe decomposition of the uronic acid groups by decarboxylation. Although it is possible to estimate the losses of individual monosaccharides by using predetermined correction factors, a more reliable approach would require reduction of the uronic acid moieties before hydrolysis.

An alternative, fast method for cleavage of glycosidic bonds in hemicelluloses with minimal degradation is based on methanolysis. In wood and pulp samples, polysaccharides are cleaved incompletely because of crystallinity and restricted accessibility in the cell wall. A complete conversion into methyl glycosides is generally brought about by heating the sample in 2 M hydrogen chloride in anhydrous methanol for 3 h at 100 °C. The hydrolysis-resistant glycosidic bonds between xylose and 4-*O*-methyl-D-glucuronic acid units in xylans are cleaved to a large extent, probably because of esterification of the uronic acid groups. Monomeric uronic acid units, which are extensively degraded in acid hydrolysis, are significantly better preserved in methanolysis

because they are converted to fairly stable methyl glycuronosides (Huang et al. 1992).

Hexenuronic acid groups linked to xylans are converted into 2-furoic acid and 5-carboxy-2-furaldehyde under acidic conditions. Although these could possibly be quantified after a total hydrolysis or methanolysis, a more convenient and reliable approach is to perform a selective hydrolysis at pH 3–4 when all other glycosidic bonds are essentially stable (Vuorinen et al. 1996).

Enzymic hydrolysis has been investigated extensively in the last two decades and applied especially to soluble hemicelluloses (Buchert et al. 1993, 1996). The use of enzymatic procedures is mainly dependent on the availability of suitable enzymic preparations, since each glycosidic bond requires its own specific enzyme to be hydrolyzed. Because the reaction conditions are mild, the hydrolysis products do not undergo undesirable secondary reactions. The enzymes currently available do not hydrolyze the glycosidic bond of hexenuronic acid groups in xylans. The hexenuronic acid groups can, however, be quantified on the basis of hexenuronic acid containing xylo-oligosaccharides (Teleman et al. 1995, 1996).

The enzymic hydrolysis of native cellulose and other cellulose-containing samples proceeds at an extremely low rate, and therefore, a pretreatment of such samples prior to hydrolysis is essential to enhance the rate of hydrolysis. In this case, the main structural features that effect the hydrolysis include the degree of crystallinity and polymerization of cellulose as well as the nature of substances with which cellulose is associated.

3.4.2 Determination of Monosaccharides

Monosaccharides in wood- or pulp-derived hydrolyzates can primarily be separated by GC, HPLC, AEC, or CE (Table 3.1). Regardless of the method applied, retention times and response factors for each monosaccharide component must be determined, usually relative to an internal standard.

Gas Chromatography. Analysis of monosaccharides by GC basically requires derivatization to increase their volatility and to decrease their affinity on the stationary phase of GC columns (Biermann and McGinnis 1989; Grant 1995). There are several well established sample preparation and derivatization methods available, as shown in Table 3.1. The most common methods are based on per(trimethylsilyl)ation of monosaccharides (or their methyl glycosides) and on the reduction of monosaccharides to alditols followed by their acetylation. In the GC analysis of monosaccharides, a flame ionization detector (FID) is commonly used.

Aldoses exist in hydrolyzates in dynamic equilibria between ring and straight chain forms (only traces of the latter). In the ring forms (pyranose and

Table 3.1. Useful methods for determination of wood monosaccharides in hydrolyzates

Separation technique	Sample preparation and derivatization	References
GC	Per(trimethylsilyl)ation	Bierman (1989a) Englmaier (1989)
	Acetylation	Fox et al. (1989) Kakehi and Honda (1989)
	Trifluoroacetylation	Neeser and Schweizer (1989) García-Raso et al. (1992) Black and Fox (1996)
	Reduction/acetylation (alditol acetates)	
	Oximation/acetylation (per-*O*-acetylated aldonitriles)	
	O-Methyloximation/acetylation or trimethylsilylation	
HPLC	Without derivatization or pre- or post-column derivatization techniques	Armstrong and Jin (1989) McGinnis et al. (1989) Akiyama (1991) Ben-Bassat and Grushka (1991) Clement et al. (1992) Del Nozal et al. (1992) Morin-Allory and Herbreteau (1992) Churms (1996a,b) El Rassi (1996) Lee (1996) Koizumi (1996)
CE	Without derivatization or pre-column derivatization techniques	Honda (1996) Linhardt and Pervin (1996) Paulus and Klockow (1996)
AEC	With and without post-column addition of NaOH	Weiss (1995) Worral and Anderson (1993)

furanose forms) two anomers (α and β) can exist, differing in the orientation of the newly formed anomeric hydroxyl group. If the anomeric center is not destroyed, derivatization freezes the anomeric equilibria, leading to two to four major components for each monosaccharide. With earlier packed columns, these components were overlapping, whereas modern capillary columns separate these mixtures without difficulty.

Reduction of aldoses with sodium borohydride (reaction for 2 h at room temperature) eliminates their anomeric center by converting them into alditols. This greatly simplifies the chromatograms, since each alditol produces only one chromatographic peak. For the *O*-methyloxime acetate procedure, the

anometric center is destroyed by a reaction with *O*-methyl hydroxylamine to generate *O*-methyloximes, followed by acetylation. However, in this reaction a new isomeric center is generated, and two derivatives are produced per aldose (*syn*- and *anti*-isomers). This means that complicated chromatograms can still result.

Table 3.2 lists the silylating reagents currently in use for derivatization of monosaccharides. These reagents are used not only alone, but also in mixtures. A simple procedure for derivatization of monosaccharides entails a combination of the reagents BSTFA (95%) and TMCS (5%). A dried sample of monosaccharides (≈1 mg), together with the internal standard (usually xylitol or mannitol), is dissolved in anhydrous pyridine (0.5 ml), and treated with the silylation reagent (0.25 ml). The mixture is shaken for approximately 30 min at room temperature before injection. Although silylation is not usually applied for analysis of mixtures of free aldoses, methyl glycosides from methanolysis are always analyzed by GC as their per(trimethylsilyl) derivatives.

High-Performance Liquid Chromatography. HPLC is a versatile method in the separation of monosaccharides and in many cases needs minimal sample pretreatment. There are many types of separation techniques and columns available. The most widely used detector for monosaccharides is a refractive index detector (RID). It should be pointed out that if a nondestructive detector such as RID is used, the separated monosaccharides may be easily recov-

Table 3.2. Silylation reagents for derivatization of monosaccharides

Reagent	Abbreviation	Structure
Trimethylchlorosilane[a]	TMCS	$(CH_3)_3SiCl$
Hexamethyldisilazane	HMDS	$(CH_3)_3SiNHSi(CH_3)_3$
N,*O*-Bis(trimethylsilyl)-trifluoroacetamide	BSTFA	$CF_3C[OSi(CH_3)_3]=N[Si(CH_3)_3]$
N,*O*-Bis(trimethylsilyl)-acetamide	BSA	$CH_3C[OSi(CH_3)_3]=N[Si(CH_3)_3]$
Trimethylsilylimidazole	TMSI	$Si(CH_3)_3(C_3H_3N_2)$
N-Methyl-*N*-(trimethylsilyl)trifluoroacetamide	MSTFA	$CF_3(CO)N(CH_3)[Si(CH_3)_3]$
N-Methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide	MTBSTFA	$CF_3(CO)N(CH_3)[Si(C_4H_9)(CH_3)_2]$
N-Trimethylsilyl-*N*-diethylamine	TMSDEA	$Si(CH_3)_3N(C_2H_5)_2$

[a] Is generally added only to accelerate silylation with other reagents.

ered. However, RID is not a particularly sensitive detector and usually a more sensitive pulsed amperometric detector (PAD) is needed. In addition, the preparation of derivatives (e.g., reaction with ethyl *p*-aminobenzoate) or various post-column derivatization techniques (e.g., reaction with ethanolamine-boric acid) allow UV/Vis or fluorescent detection.

Other Separation Techniques. Recently, CE was applied for separation of monosaccharides. In this method a relatively good resolution of monomeric components (both monosaccharides and uronic acids) can be achieved. Detection of nonderivatized monosaccharides is generally based on direct and indirect UV, amperometric, and RI detections, whereas UV and fluorescence detections are applied to derivatized monosaccharides. A variety of monosaccharides can also be separated by AEC under isocratic conditions using a dilute sodium hydroxide solution as eluent. In this method, PAD is generally used for detection.

3.5 Linkage Positions

Determination of the linkage positions in polysaccharides is based mainly on permethylation analysis (Jansson et al. 1976; Aspinall 1982b; York et al. 1985). After methylation of the free hydroxyl groups, the polysaccharide is hydrolyzed or methanolyzed (Fig. 3.8). Simultaneously, information on anomeric configuration and ring-size is lost. Glycosidic bonds can also be reductively cleaved with retention of ring-size but in this case the asymmetry at the anomeric carbon atom disappears.

Linkage positions in oligo- and polysaccharides can also be determined by novel multidimensional NMR techniques, which are generally used in a sequence analysis (Vliegenthart 1997). Analysis of linkage positions by NMR does not necessarily require much sample preparation, but the NMR techniques are much more demanding than GC/mass spectrometry (GC/MS) applied for the separation and identification of partially methylated monosaccharides.

3.5.1 Permethylation

The permethylation of polysaccharides is usually performed according to Hakomori's procedure, where the sample is dissolved in dimethyl sulfoxide, and the methylation brought about by methyl iodide with methyl sulfinyl ion as the base catalyst (Hakomori 1964). Esters, reducing sugar units, and even uronic acid structures are labile under the strongly basic conditions

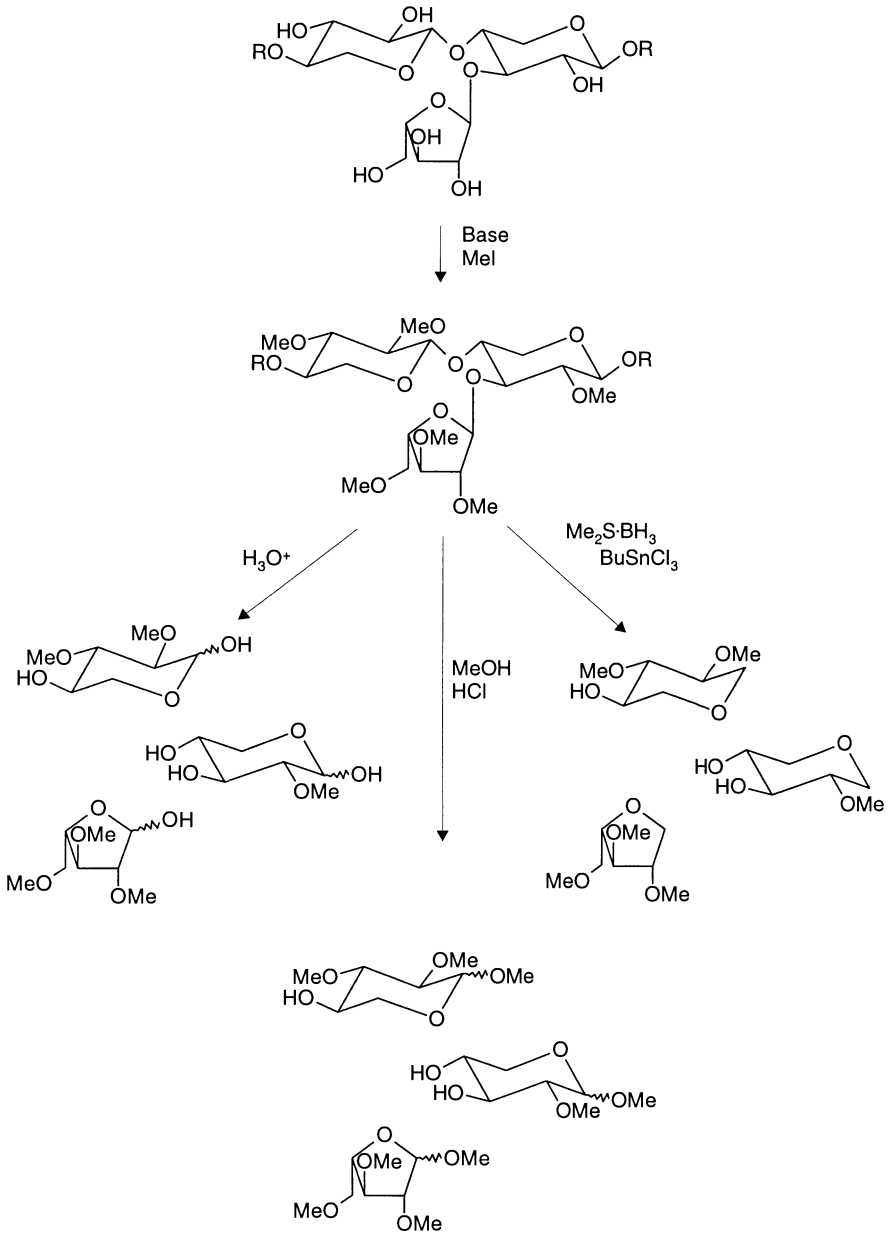

Fig. 3.8. Principle of determination of linkage positions by permethylation analysis

applied. Alternatively, polysaccharides can be permethylated with methyl trifluoromethanesulfonate under mildly basic conditions according to Prehm's method. Although all structures, including esters, are stable under these conditions, the applicability of Prehm's procedure is limited by the low solubility of polysaccharides in the trimethyl phosphate used as the solvent (Prehm 1980).

3.5.1.1 Methylation with Methyl Iodide

Methylation with methyl iodide requires strongly basic conditions. In Hakomori's method, dimethyl sulfinyl (dimsyl) ions (prepared from butyllithium in anhydrous dimethyl sulfoxide) serve as the base catalyst (Kvernheim 1987). If the solubility is limited, the sample can be premethylated with methyl iodide in aqueous sodium hydroxide. The premethylated polysaccharides are soluble in dimethyl sulfoxide and can be permethylated according to Hakomori's method.

Because of the strongly alkaline conditions applied, Hakomori's method would lead to degradation of reducing end groups, which therefore must be protected by reduction with sodium borohydride to alditol end groups. Esterification of carboxylic acid groups facilitates a fast β-elimination of uronic acid groups. Although the resulting hexenuronic acid groups may partially remain on the permethylated polysaccharide, they are degraded during the hydrolysis (or methanolysis). Therefore, carboxylic acids must be reduced before the methylation.

In one procedure, uronic acid groups are activated by a water-soluble carbodiimide before their reduction with sodium borohydride (Taylor and Conrad 1972; Fig. 3.9). Because the reduction products of uronic acid groups are inseparable from the corresponding aldoses, reduction with sodium borodeuteride is frequently applied. Labeling with deuterium enables identification and quantification of uronic acids by MS.

3.5.1.2 Methylation with Methyl Trifluoromethanesulfonate

Methylation of carbohydrates follows the S_N2 mechanism and is thus affected by the leaving group ability of the methylating agent. Trifluoromethanesulfonoxy (triflyloxy) substituent is a very good leaving group in comparison with iodide, and methylation with methyl trifluoromethanesulfonate (methyl triflate) therefore proceeds much faster than with methyl iodide. Because of the rapid reaction, only a weak base catalyst is needed. Prehm's method applies 2,6-di-*tert*-butylpyridine as the base catalyst. Under the conditions applied, even ester groups survive the treatment (Prehm 1980).

Polar aprotic solvents, such as dimethyl sulfoxide, diminish the reactivity of methyl triflate. In Prehm's method, trimethyl phosphate is used as the solvent.

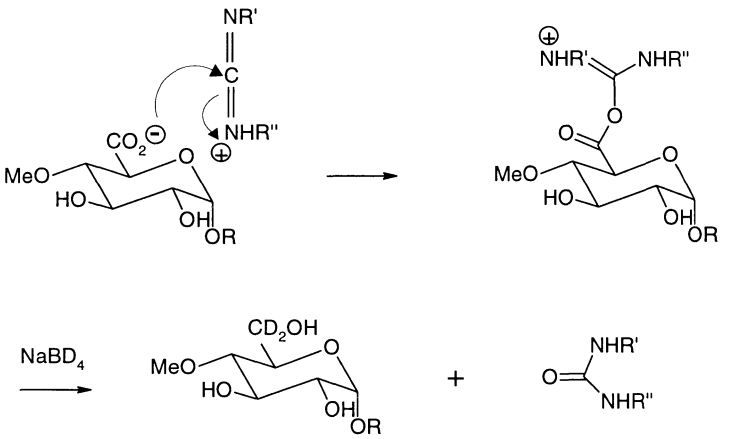

Fig. 3.9. Reduction of carbodiimide-activated 4-*O*-methylglucuronic acid group by sodium borodeuteride to a deuterated 4-*O*-methylglucopyranosyl group

Although the solubility of anionic polysaccharides in trimethyl phosphate can be increased by changing their counter ions to triethylammonium, the low solubility generally limits the applicability of Prehm's method for their permethylation.

Unlike polysaccharides, most oligosaccharides are readily soluble in trimethyl phosphate and Prehm's method is therefore well suited for permethylation analysis of oligosaccharides generated from polysaccharides for the purpose of sequence analysis. An advantage is that the reducing end group of the oligosaccharides does not need to be protected before methylation when methyl triflate is used as the methylating agent.

3.5.1.3 Techniques for *O*-Acylated Polysaccharides

Although *O*-acylated polysaccharides can be methylated with methyl triflate without deacylation, the acyl groups are cleaved during hydrolysis (or methanolysis) of the permethylated polysaccharide. Determination of the site of the acyl groups would thus require deacylation by base catalysis and subsequent ethylation with ethyl iodide or ethyl triflate.

In another approach, a completely different strategy is used for the determination of the sites of *O*-acylation. The *O*-acylated polysaccharide is treated first with methyl vinyl ether under mild acid conditions to convert the free hydroxyl groups to acetals (Fig. 3.10). In the second step, the acetal protected polysaccharide is treated under Hakomori's conditions. The acetals are stable but deacylation leads to liberation of hydroxyl groups, which are readily methylated. In a hydrolysis step both glycosidic bonds and the ethylmethyl acetals are cleaved, resulting in monomers that have *O*-methyl

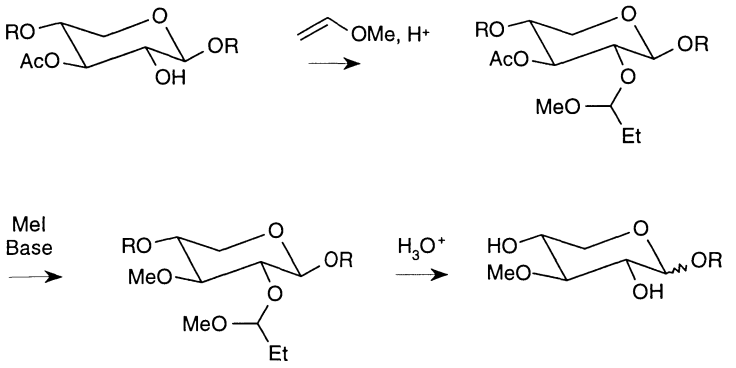

Fig. 3.10. Determination of sites for *O*-acylation by permethylation analysis. In this modification, free hydroxyl groups are protected by acetal formation and hence only *O*-acyl groups are methylated

groups on the sites of the original *O*-acyl groups (De Belder and Norrman 1968).

3.5.2 Cleavage of Glycosidic Bonds

Polysaccharides methylated by Hakomori's method are usually purified by dialysis. With Prehm's procedure the permethylated product is recovered by extraction with chloroform-water and purification (organic layer) by GPC. The purification by GPC can also be applied to permethylated oligosaccharides. Glycosidic bonds in the purified product are hydrolyzed, methanolyzed, or cleaved reductively. Permethylated polysaccharides are insoluble in water and are therefore generally hydrolyzed with volatile organic acids, including formic or trifluoroacetic acid.

Methanolysis is well suited for the depolymerization of permethylated polysaccharides that are completely soluble in methanol. All glycosidic bonds, including glycuronosidic bonds, are smoothly cleaved. *O*-Acylated polysaccharides are deacylated and uronic acids are converted into their methyl esters. Information about anomeric configuration is lost, and each sugar unit gives an equilibrium mixture of α- and β-glycosides. 4-*O*-Linked pyranosides and 5-*O*-linked furanosides yield mixtures of methyl furanosides and pyranosides, and information on the ring size is lost (Fig. 3.11). In other cases, the ring size is retained. Thus, depending on linkage positions, each sugar unit gives two or four stable monomeric structures.

In the presence of a Lewis acid catalyst, permethylated glycosides can be hydrogenated to corresponding anhydroalditols. The ring size is retained but anomeric information is lost. Aldofuranosides and -pyranosides yield only one

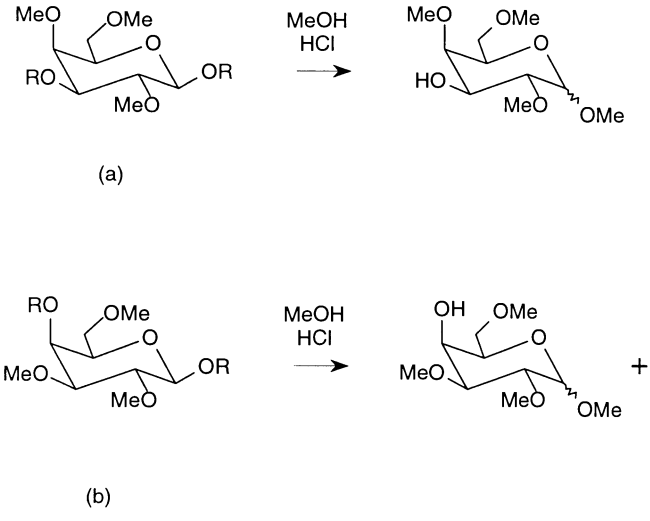

Fig. 3.11. Methanolysis of permethylated **a** 1,3-galactan and **b** 1,4-galactan. Anomerization prevents determination of the actual bonding site in the latter case

product, whereas ketofuranosides and -pyranosides give an epimeric pair of products. A very selective and quantitative reduction can be brought about with borane-methyl sulfide when butyltin trichloride is used as a catalyst (Wang and Gray 1997).

3.5.3 Separation of Monomers by GC

The monomers from permethylated polysaccharides cannot usually be separated as such by GC. The free hydroxyl groups in the derivatives (corresponding to the sites of the interunit linkages in the original polysaccharide) must first be substituted with a suitable hydrophobic group in order to increase the volatility. The methyl glycosides, obtained by methanolysis of a permethylated polysaccharide, are generally converted into their per(trimethylsilyl) derivatives, whereas the anhydroalditols from a reductive cleavage are peracetylated.

Although the product mixtures after methanolysis are complex, a good resolution on GC capillary columns can be achieved even when mixtures of hemicelluloses are analyzed (Fig. 3.12). The retention times on the unpolar columns generally used decrease in the order of increasing degree of methylation.

The free partially methylated aldoses, obtained after hydrolysis of the permethylated polysaccharides, are almost always reduced to the corresponding alditols and acetylated. The reduction is often performed with sodium borodeuteride so that the original anomeric carbon atom can be identified by

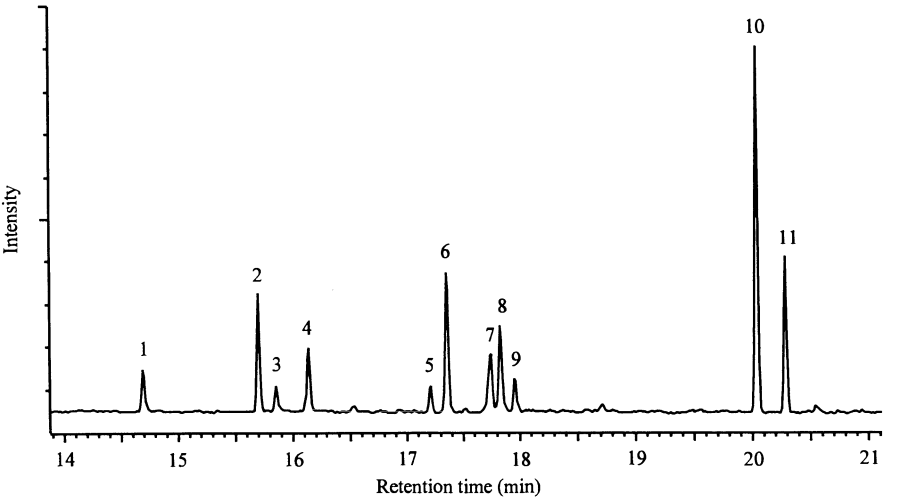

Fig. 3.12. Separation of partially methylated methyl glycosides, liberated from a permethylated glucogalactan, by GC. Methyl glycosides: 2,3,4,6-tetra-*O*-methylglucopyranosides (*1,2*), 2,3,4,6-tetra-*O*-methylgalactopyranosides (*3,4*), 2,3,6-tri-*O*-methylgalactopyranosides (*5,7*), 2,3,6-tri-*O*-methylgalactofuranoside (*6*), 2,4,6-tri-*O*-methylgalactopyranosides (*8,9*), 2,4-di-*O*-methylgalactopyranosides (*10,11*)

MS. Relative retention times of partially methylated alditol acetates on several stationary phases have been tabulated (Jansson et al. 1976).

3.5.4 Structure of Monomers by GC/MS

Identification of the partially methylated monosaccharides or their derivatives is based on selective fragmentation in electron impact (EI) MS (Lönngren and Svensson 1974). Although the molecular ion is not always found, the fragmentation pattern, together with retention time on GC columns, reveals the substitution pattern. Mass spectra of stereoisomers are practically identical and their differentiation must therefore be based on relative retention times on GC columns.

In acyclic, partially methylated alditol acetates, primary fragmentation occurs as fission of carbon-carbon bonds (Fig. 3.13). Linkages between two *O*-methylated carbon atoms are most easily broken, whereas bonds between two *O*-acetylated carbon atoms are most stable. The positive charge is likely localized on an *O*-methylated fragment.

The primary fragments are partly converted to secondary fragments by β-elimination of acetic acid and methanol or α-elimination of acetic acid. Additional fragments are formed through elimination of ketene and formaldehyde.

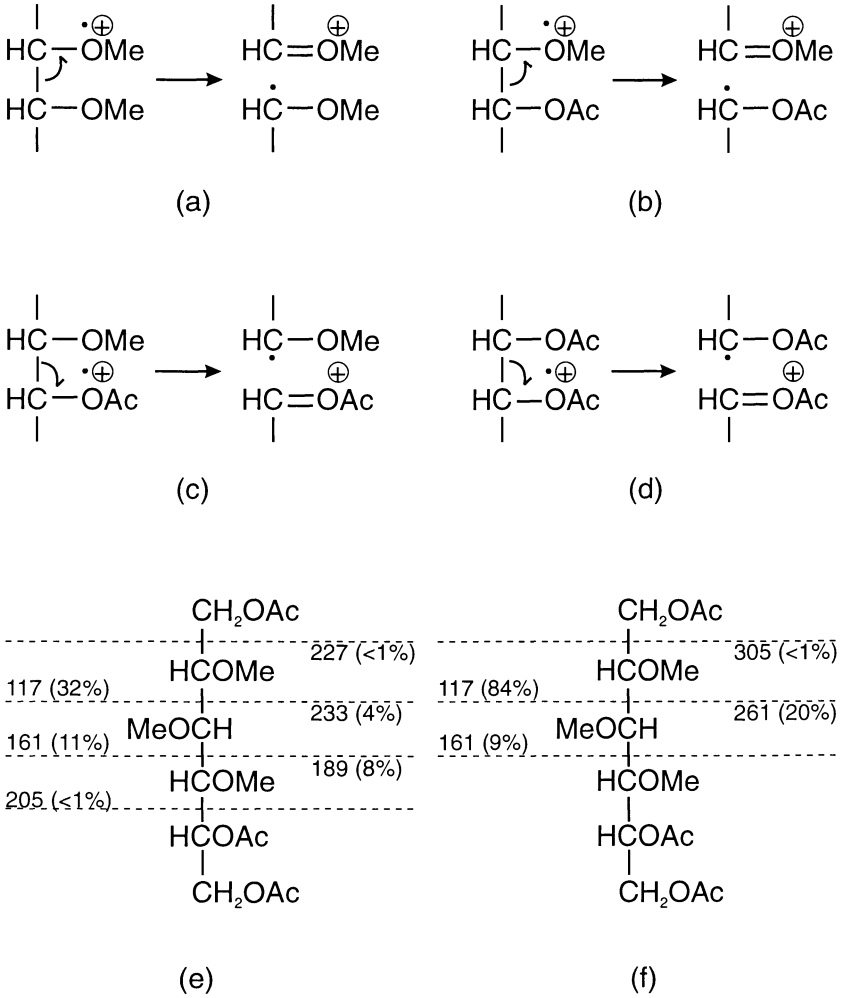

Fig. 3.13. Fragmentation patterns of partially methylated alditol acetates in EI-MS. Note that the fragmentation mechanism **a** is most probable followed by **b**. (Lönngren and Svensson 1974)

Trimethylsilyl derivatives of partially methylated methyl pyranosides give three characteristic fragments, from which the substitution pattern at C-2, C-3, and C-4 can be assigned (Petersson 1975; Fig. 3.14). Possible *O*-methylation at C-6 is difficult to show directly, although its presence may be indicated by the absence of a fragment from an *O*-trimethylsilylated hydroxymethyl group (m/z 103). This can be confirmed by retention on GC columns because an additional *O*-methylation always shortens the retention time.

A single fragment, carrying the *O*-substituents at C-2 and C-3, dominates the spectra of furanosides. An additional fragment $CH(OR_1)(OR_2)^+$, similar to

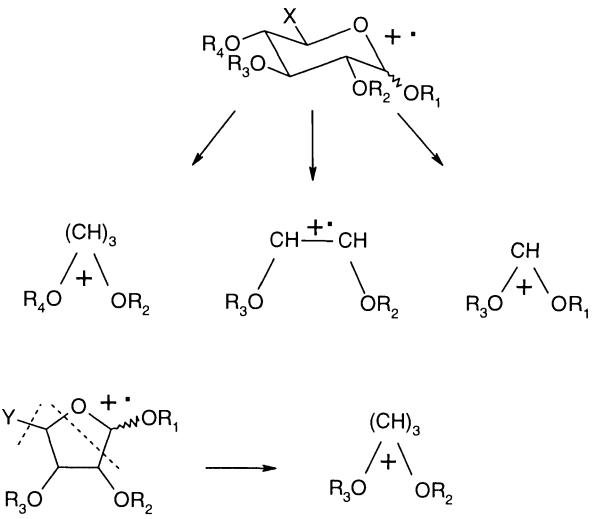

Fig. 3.14. Fragmentation patterns of trimethylsilyl derivatives of partially methylated methyl glycosides by EI-MS. (Petersson 1975)

that in the spectra of pyranosides, can be used to determine whether a possible *O*-methyl group is located at C-2 or C-3. Fragmentation in the side chain at C-4 reveals a possible occurrence of *O*-methyl groups at C-5 and C-6.

3.6 Linkage Sequence

Although multidimensional NMR techniques can be used for determination of bonding sites, a complete sequence analysis of unmodified polysaccharides is still not possible. NMR signals from related structural environments are overlapping, especially because the low thermal mobility of polysaccharides leads to line broadening (Vliegenthart 1997).

The sequence analysis of polysaccharides is based on their partial hydrolysis and on the structural analysis of the oligosaccharides liberated. Because of the randomness of partial hydrolysis, a certain structural feature in the polysaccharide exists in several oligosaccharides, which can be used for reconstructing of the polymer from the oligomers (Fig. 3.15).

In many cases, selective hydrolysis can give valuable information on the structure of the polysaccharide, especially when it has several structural domains. Selective chemical hydrolysis is limited to a few structures, whereas selective enzymic hydrolysis can be applied to almost all possible linkages in polysaccharides (White and Kennedy 1988b).

Both chemical and enzymic hydrolysis generate mixtures of oligosaccharides. Tracing of the structures of oligosaccharides in mixtures is possible espe-

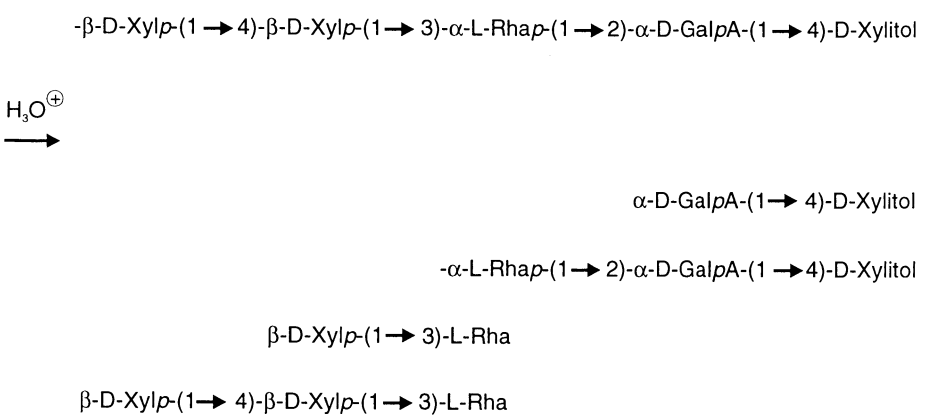

Fig. 3.15. Principle of the analysis of linkage sequence of polysaccharide through its partial hydrolysis. The (sub)structure of the polysaccharide is reconstructed from overlapping segments of oligosaccharides

cially by tandem FAB-MS, but generally pure oligosaccharides must be isolated before structural analysis by NMR or MS (Hicks 1988). Separation of the oligosaccharides is usually based on molecular weight and charge. Certain sugar units may have specific interactions with metal ions or oxy anions, which can also be applied to purification by column chromatography.

3.6.1 Partial Hydrolysis

3.6.1.1 Chemical Hydrolysis

Among the native glycosidic bonds in plant polysaccharides, furanosidic linkages are most labile toward acids (Overend 1972). The activation energy for the hydrolysis of furanosides is lower than for pyranosides and therefore the most selective hydrolysis is performed at a low temperature and low pH rather than at a higher temperature and a higher pH.

Hexenuronic acid groups, which are formed from 4-*O*-substituted hexuronic acids by alkali, are also liberated under mild conditions. The pH dependence is different from that of general glycoside hydrolysis, and the most selective hydrolysis occurs at pH 3–4 (Vuorinen et al. 1996).

The relative hydrolysis rates between pyranosides are not very different. Glycuronosides which are much more stable toward acids than neutral pyranosides are an exception. Acetolysis favors glycosidic bond cleavage in structures that are bound to primary hydroxyl groups. Thus, acetolysis cleaves selectively 1,6-linked pyranosides.

Glycosidic bonds are generally stable toward bases. However, 1,4-linked galacturonosides undergo β-elimination under mild conditions, especially when the uronic acid groups are esterified (Kiss 1974). The unit that undergoes β-elimination is converted into a hexenuronic acid group.

3.6.1.2 Enzymic Hydrolysis

Enzymes hydrolyzing glycosidic bonds can be divided into endo- and exoglycanases (White and Kennedy 1988b; Buchert et al. 1996; Fig. 3.16). Endoglycanases cleave polysaccharide chains within nonterminal units, whereas exoglycanases remove one or two sugar units from the end of the polymer. Glycanases are highly specific with respect to configuration, absolute configuration (D- and L-sugars), ring size, and anomeric configuration of sugar units. Glycanases may also show selectivity or even specificity toward the environment where the sugar unit exists. Native *O*-acetyl groups in plant polysaccharides can be hydrolyzed with esterases.

Glycosidases, a subgroup of exoglycanases, remove terminal, nonreducing sugar units almost irrespective of the binding site of the sugar unit. Glycosidases are typically applied for debranching of polysaccharides, although longer homopolymer fragments could also be cleaved by subsequent hydrolysis of several terminal units.

Although endoglycanases act on single glycosidic bonds, substituents at close proximity of the bond may prevent hydrolysis. The effect of substituents on enzyme activity depends on the type of the enzyme. Typically, endoglycanases act only on subregions with at least two or three vicinal, unsubstituted sugar units. Some endoglycanases are active only toward structures having a specific substituent. Thus, a glucuronoxylanase hydrolyses xylan only at a point of a 4-*O*-methyl-D-glucuronic acid substituent.

3.6.2 Fractionation of Oligosaccharides

Partial hydrolysis of polysaccharides never yields pure oligosaccharides but mixtures of several, often structurally related oligosaccharides. Separation of pure fractions from these mixtures is demanding and it can be the most laborious stage in sequence analysis. Acidic oligosaccharides can be fractionated with AEC or CE according to their charge (Hicks 1988; Rydlund and Dahlman 1997; Fig. 3.17). Neutral oligosaccharides can also be charged by ionization of hydroxyl groups or by formation of adition compounds with borate ions.

Gel permeation chromatography (GPC) separates oligosaccharides according to their hydrodynamic volume. The hydrodynamic volume is not only related to molecular weight but also to conformation and substitution of the oligosaccharide. The conformation depends mainly on favored rotamers

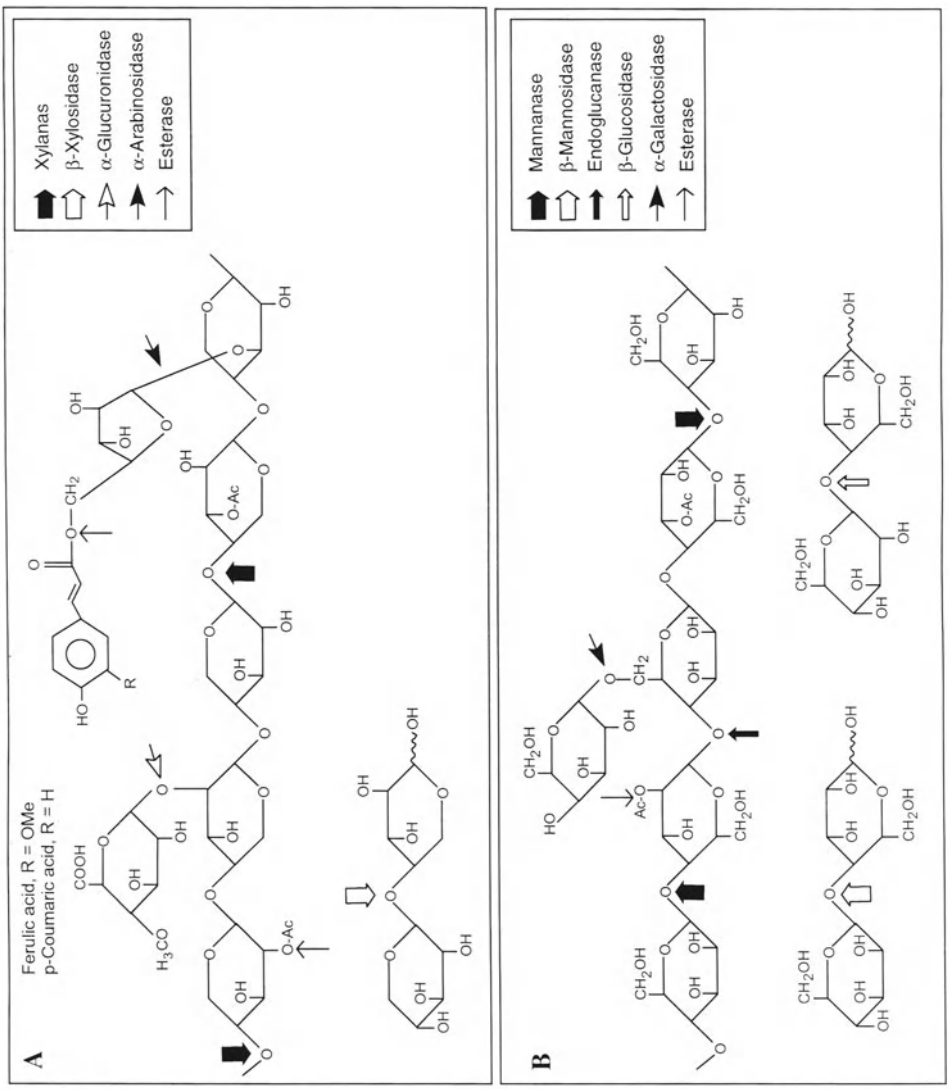

Fig. 3.16. Specific enzymes for partial hydrolysis of **A** plant cell wall xylans and **B** galactoglucomannans. Note that all xylans do not necessarily contain the substructures shown in scheme **A**

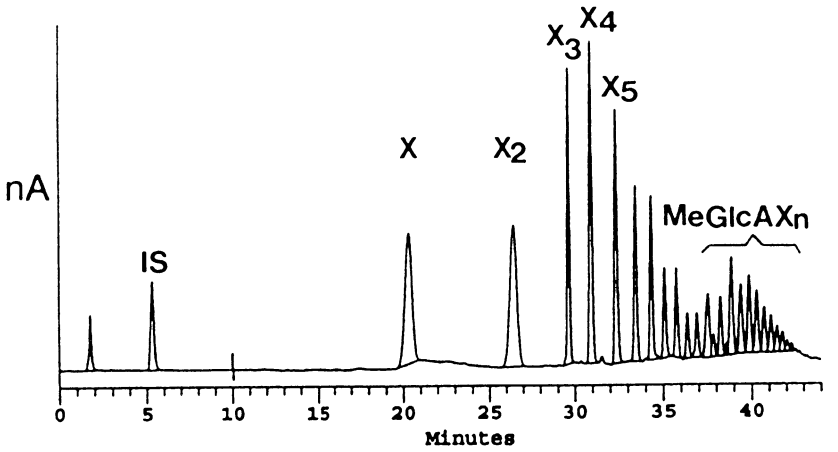

Fig. 3.17. Fractionation of xylo-oligosaccharides, obtained by hydrolysis of a *O*-acetylglucuronoxylan by an endo-xylanase, by AEC. Neutral xylo-oligosaccharides are eluted in the order of increasing molecular weight followed by acidic xylo-oligosaccharides

around carbon-oxygen bridges between sugar units. Although the resolution of GPC columns facilitates separation of homologous oligosaccharides, it is clear that a similar separation of structurally different oligosaccharides is impossible.

Alkaline earth metal ions form relatively strong complexes with vicinal *cis*-diols. In wood polysaccharides, free vicinal *cis*-diols exist in 1,4-linked mannose units and terminal or 1,6-linked galactose units. Fractionation of the corresponding oligosaccharides according to their mannose or galactose content can be based on chromatography on cation exchange resins in Ca^{2+}, Ba^{2+}, or La^{2+} forms.

3.6.3 Structure of Oligosaccharides

3.6.3.1 Fast Atom Bombardment Mass Spectroscopy

A key step in MS analysis of oligosaccharides is the ionization. EI leads to severe fragmentation of sugar units and is suitable mainly for structural analysis of monosaccharides and their derivatives. At the other end, matrix assisted laser desorption ionization (MALDI) yields mainly molecular ions from oligo- and polysaccharides and is ideal for the analysis of molecular weight. In sequence analysis of oligosaccharides, fragmentation should ideally take place only at carbon-oxygen bonds between sugar units. This requirement is best

fulfilled by fast atom bombardment MS (FAB-MS), although not with complete selectivity (Dell 1987).

FAB-MS, like MS techniques generally, does not differentiate well between stereoisomers, and sugar monomers can therefore be identified only according to their molecular weight. Thus, the structural units detected by FAB-MS are pentoses, hexoses, hexuronic acids, *O*-methyl hexuronic acids, etc. Primary fragmentations give ions that correspond to all possible mono- and oligosaccharides that could be formed from the parent oligosaccharide by cleavage of one glycosidic bond (Fig. 3.18). It follows from this that FAB mass spectra of linear and branched oligosaccharides are different. FAB-MS does not, however, show at which carbon atoms the glycosyl substituents are located. Interpretation of FAB mass spectra can be simplified by some kind of derivatization of the reducing sugar unit, which firmly labels one end of the parent and daughter molecules.

When FAB is combined with tandem MS (MS-MS), the determination of sequences from oligosaccharide mixtures becomes possible. In principle, the second MS (which should be FAB-MS) is used to study the secondary fragmentation of single molecular or fragment ions from the first MS. The method is very useful for mixtures of oligosaccharides of varying molecular weights.

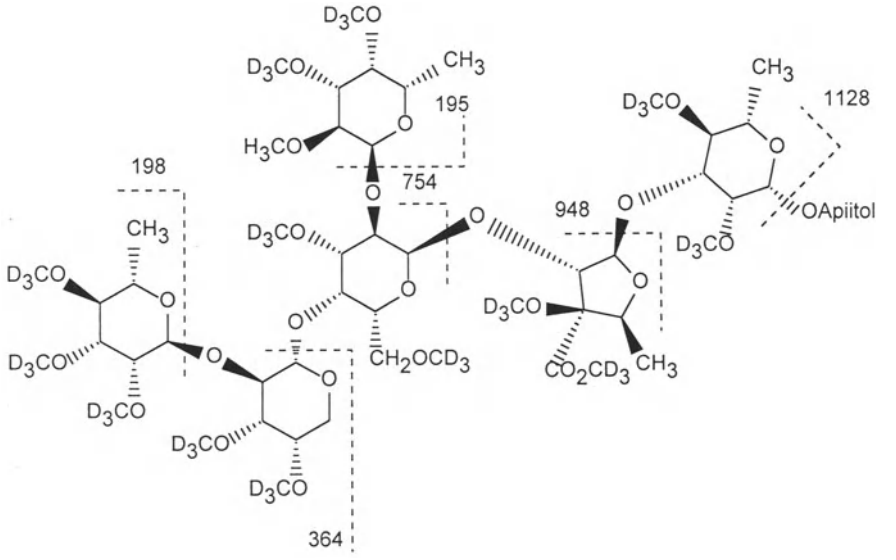

Fig. 3.18. Fragmentation pattern of a complex per-*O*-(trideuteriomethyl)ated heptasaccharide-alditol by FAB-MS. The oligosaccharide is a substructure of rhamnogalacturonan II of the plant cell wall. Adopted from Spellman et al. 1983

3.6.3.2 Nuclear Magnetic Resonance Spectroscopy

The structure of pure oligosaccharides can often be completely determined by using multidimensional NMR spectroscopy. Because the NMR spectra of α- and β-anomers of free oligosaccharides are unequivalent, they are usually reduced with sodium borohydride before analysis. Although the reduction of the reducing end group to an alditol end group simplifies the NMR spectrum, signals from nonanomeric protons are heavily overlapping. Any attempts to identify the structure by one-dimensional techniques would be hopeless.

Identification of individual sugar monomers of an oligosaccharide is done mainly by total correlation spectroscopy (TOCSY; van Halbeek 1994; Vliegenthart 1997). It detects interactions between nuclei through covalent bonds and shows chemical shifts and couplings in full, isolated spin systems. In oligosaccharides, carbon protons of each monosaccharide unit form a spin system that is isolated from the spin systems of neighboring monosaccharide units by interunit carbon-oxygen bonds. Hence, TOCSY separately shows clean one-dimensional ^{1}H NMR spectra for all monosaccharide units (Fig. 3.19).

The sequence itself is determined by NOE (nuclear Overhauser effect) spectroscopy (NOESY) or rotating-frame NOE spectroscopy (ROESY). These techniques measure the magnitude of dipolar interactions between nuclei through

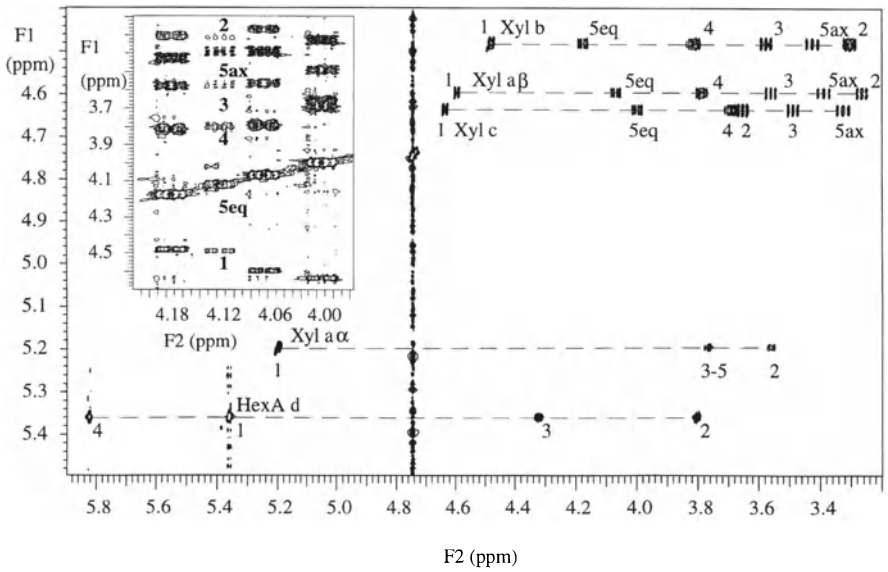

Fig. 3.19. TOCSY spectrum of a mixture of xylo-oligosaccharides derived from an alkali-treated 4-*O*-methylglucuronoxylan, showing subspectra of the hexenuronic acid group (*HexA*) and reducing and nonreducing xylose units in several chemical environments. (Teleman et al. 1996)

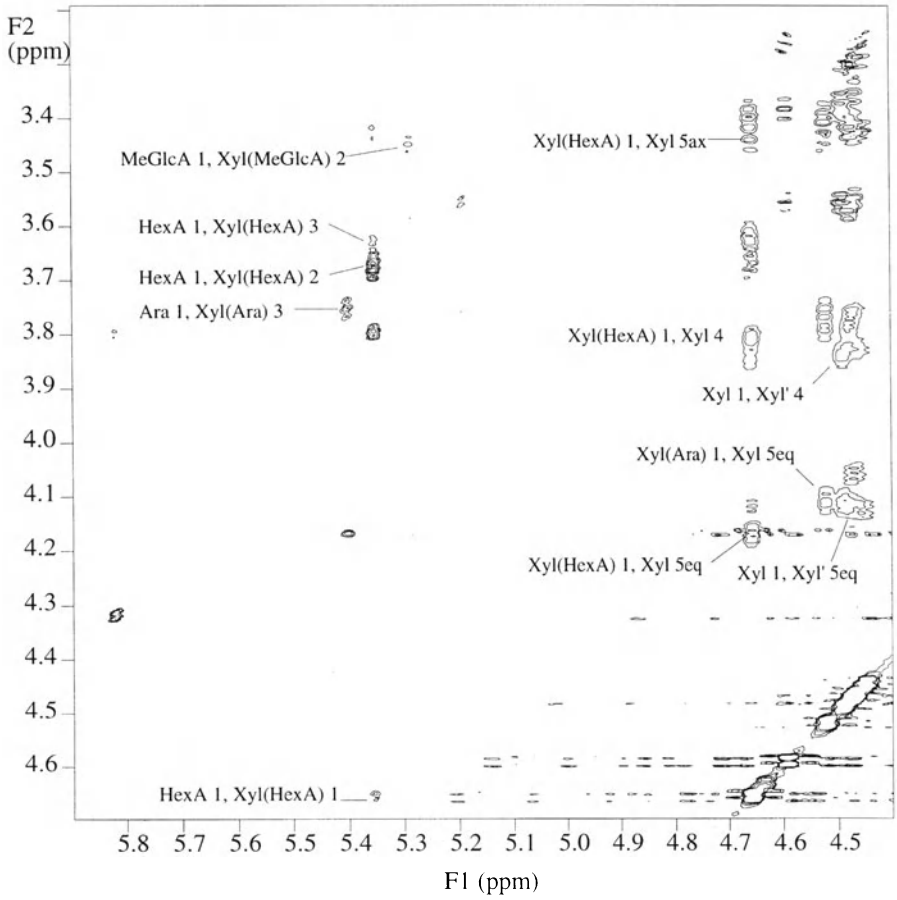

Fig. 3.20. NOESY spectrum of a mixture of acidic xylo-oligosaccharides obtained from a softwood kraft pulp through enzymic hydrolysis and AEC, showing NOE cross-peaks of anomeric protons of hexenuronic acid (*HexA*), arabinose, and xylose units with nonanomeric protons of neighboring xylose units. (Teleman et al. 1995)

space. The intensity of NOE is inversely proportional to the sixth power between nuclei and through-space interactions are therefore detected only at short distances (<1 nm). In NOESY (or ROESY), strong cross-peaks of an anomeric proton with other protons reveal not only the neighboring monosaccharide unit but also the bonding site at this unit (Fig. 3.20).

3.7 Anomeric Configuration and Ring Size

In the previous sections we have seen that the ring size of monosaccharide units in permethylated polysaccharides can be determined by analysis of prod-

ucts after a reductive cleavage. The activity of specific endo- and exoglycanases may indirectly define both ring size and anomeric configuration. Acids hydrolyze furanosides much faster than pyranosides, which can also serve as indirect evidence on ring size. Generally, information on both ring size and anomeric configuration is, however, obtained by NMR spectroscopy (van Halbeek 1994).

^{1}H NMR spectra of individual monosaccharide units, obtained by TOCSY or other NMR techniques, in principle define their structure. Often ^{1}H NMR data are complemented with ^{13}C NMR measurements using heteronuclear correlation spectroscopy. Chemical shifts and coupling constants for a multitude of mono- and oligosaccharides have been reported and collected in libraries. Software is also available for computer aided identification of the absolute structure from experimental NMR data.

Identification of the anomeric configuration by NMR can be based on ^{1}H chemical shifts, ^{13}C chemical shifts, vicinal coupling of anomeric proton to H-2 ($^{3}J_{H1,H2}$), or coupling between anomeric proton and anomeric carbon ($^{1}J_{H1,C1}$) (Bock and Thögersen 1982; Perlin and Casu 1982; Bock and Pedersen 1983; Tvaroska and Taravel 1995; Fig. 3.21). The chemical shift of an anomeric proton in pyranosides depends mainly on the orientation of the anomeric oxygen (or proton). Axial anomeric protons (β-D-pyranoses and β-D-pyranosides in $^{4}C_{1}$ conformation) resonate at 4.3–4.7 ppm, whereas equatorial anomeric protons (α-D-pyranoses and α-D-pyranosides in $^{4}C_{1}$ conformation) appear at 4.9–5.6 ppm.

The vicinal coupling $^{3}J_{H1,H2}$ is large (7–8 Hz) when both H-1 and H-2 are axial, which can be applied fo firmly assign the anomeric configuration to β (D-pyranosides in $^{4}C_{1}$ conformation). Coupling between an axial and an equatorial or two equatorial protons is weaker (1–4 Hz). Coupling between an axial anomeric proton (β-D-pyranosides in $^{4}C_{1}$ conformation) and the anomeric carbon is weaker ($^{1}J_{H1,C1} \approx 160$ Hz) than coupling between an equatorial anomeric proton (α-D-pyranosides in $^{4}C_{1}$ conformation) and the anomeric carbon atom ($^{1}J_{H1,C1} \approx 170$ Hz).

Anomeric carbon resonances at a high field ($\delta_{C1} \approx 100$ ppm) are indicative of pyranosides with an axial anomeric substituent. Anomeric carbon atoms of

	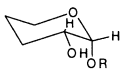	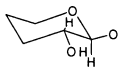	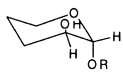	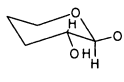	
δ_{C1} (ppm)	100 - 101	104 - 105	102	101	103 - 110
δ_{H1} (ppm)	4.7	4.2 - 4.3	4.6 - 4.7	4.4 - 4.5	4.9 - 5.0
$^{1}J_{C1H1}$ (Hz)	170	160	170	160	172 - 174
$^{3}J_{H1H2}$ (Hz)	3 - 4	7 - 8	1 - 2	1 - 2	1 - 5

Fig. 3.21. ^{1}H and ^{13}C NMR parameters of anomeric pyranosides and furanosides

other pyranosides resonate typically at 101–105 ppm. Resonances at especially low fields ($\delta_{C1} \approx 110$ ppm) are characteristic of several furanosides.

The nonanomeric carbon atoms of unsubstituted pento- and hexopyranosides have chemical shifts at 67–77 ppm except for the terminal carbon atom that resonates at 60–67 ppm. A glycosidic substitution at a carbon atom increases its chemical shift by ≈10 ppm and the chemical shift of the proton attached to it by ≈0.5 ppm, both of which can be used for determination of linkage positions. In furanosides, C-4, and sometimes also C-2, resonates at a low field (79–86 ppm), which is indicative but can be interfered with by substitution of pyranosides at C-4.

3.8 Molecular Weight

Because plant polysaccharides are polydisperse, it is usually of interest to determine their molecular weight distribution rather than the average molecular weight. Today, molecular weight distributions of polysaccharides are mainly determined by GPC. New ionization methods, including MALDI, make it possible to analyze molecular weight distributions also by MS.

Some of the most important reactions of wood polysaccharides during pulping and bleaching occur at the reducing end groups, which has emphasized the importance of an end group analysis. The molecular weight of cellulose is usually followed by its viscosity and also by some GPC detectors, which measure viscosity directly (Fishman et al. 1993). Because the viscosity of a polymer depends on its shape, light scattering techniques are more reliable for determining the true molecular weights of both cellulose and hemicelluloses.

3.8.1 Gel Permeation Chromatography

In GPC, often also called size exclusion chromatography (SEC), molecules are separated according to their ability to penetrate into a porous chromatography gel. The penetration depends on hydrodynamic volume or radius of the molecules rather than their molecular weight. Molecular weight calibration of GPC columns is usually performed with globular proteins or close to monodisperse fractions of dextrans or pullulans of known molecular weight. Strictly speaking, this kind of calibration is valid only for the same polymers. There is much variation in conformation or shape of polysaccharides and, for example, structural polysaccharides in plant cell walls form a loose random coil, quite different from the tight globular structure of proteins.

Many polysaccharides are insoluble in water and therefore other eluents are frequently needed for their separation. Solutions of sodium hydroxide are generally applied as eluents in GPC of xylans. Chemical pulps are often com-

pletely dissolved in lithium chloride/dimethyl acetamide that can be used as an eluent in the separation of dissolved polysaccharides by GPC (Timpa 1993; Striegel and Timpa 1995). Agarose and dextran based GPC gels are generally applied for separation of aqueous polysaccharide samples. These gels are also compatible with dilute solutions of sodium hydroxide.

The elution of polysaccharides is usually followed by a refractometer or by a post-column reactor with, for example, phenol-sulfuric acid test, periodate oxidation, or total organic carbon determination. The molecular weight can be evaluated on-line indirectly by viscometry or directly by light scattering.

3.8.2 End Group Analysis

Native plant polysaccharides generally have reducing end groups. However, during kraft pulping they are largely converted to deoxyaldonic and partly to aldonic acid end groups. The determination of the type and number of reducing end groups is based on their reduction to corresponding alditols by sodium borohydride. After total hydrolysis, the alditols are separated from aldoses by chromatography on a strongly basic anion exchange resin in the hydroxyl form. Due to their lower acidity, alditols are eluted with water earlier than the aldoses.

The acid end groups are also liberated during total hydrolysis of the polysaccharide. In the hydrolyzate they exist partly as lactones. Separation of the acids is effected by chromatography on an anion exchange resin in acetate form. Because interconversion between (deoxy)aldonic acids and their lactones is slow under neutral or slightly acidic conditions, the lactones must be opened by alkali before the separation. If an analytical column is used, the (deoxy)aldonic acids can be separated from one another by using sodium acetate buffer as the eluent. For group separation, acetic acid can also be used as the eluent.

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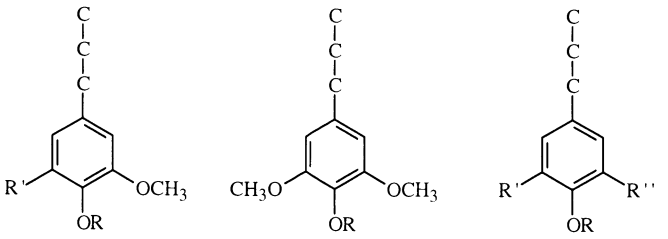
4 Lignin

G. Brunow, K. Lundquist, and G. Gellerstedt

4.1 Definition

The term *lignin* is commonly used for unchanged lignin as well as for lignin-derived materials in pulps and pulping liquors. This meaning of the term lignin is retained in this chapter. However, analytical data for unchanged lignin are usually not valid for lignin-derived materials in pulps and pulping liquors because of chemical modifications. Furthermore, analytical techniques used for the examination of unchanged lignins are not always applicable for the examination of chemically modified lignins. Therefore we use the term *protolignin(s)* (native lignin(s)) when we specifically refer to unchanged or essentially unchanged lignin(s).

Protolignins are biopolymers occurring in vascular plants as one of the major structural components. The building blocks in lignins are phenylpropane units of types **1–3**. However, a small proportion of the units can be considered as conversion products of units **1–3** and occasionally a few other types of building blocks are also present in protolignins (see below). The structures of protolignins are irregular in the sense that different structural elements are not linked to each other in any particular order.



1 **2** **3**

R = H or C
R' = H, C or O
R''= H, C or O

The proportions of units **1–3** in protolignins vary with their botanical origin. The biological microstructure of wood and the presence of linkages between the structural polymers together with the high molecular weight of the wood constituents prevent the quantitative isolation of the protolignin component of wood in a completely unchanged form. This makes it difficult to study protolignins and also to define them chemically. However, it is evident

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that protolignins constitute a class of biopolymers that distinctly differ in structure from other classes of biopolymers.

Chemical compounds are usually defined by a precise structural formula together with some complementary data (e.g., physical constants such as melting point, crystal structure data, and hydrogen bonding). The structural formula could be said to summarize the results of analytical examinations. It is not possible to define protolignins in this manner. In an early attempt to circumvent this difficulty it was suggested that protolignins should be defined as polymeric materials that give rise to so-called Hibbert ketones on refluxing with hydrogen chloride in ethanol (Fig. 4.1; Brauns and Brauns 1960). Such degradation products, which are readily detected, originate from a major type of structural element in protolignins, the β-*O*-4 structure (see below), and exhibit sufficient structural complexity to make protolignins (and no other polyphenol) the only reasonable source for their formation.

Today several lignin degradation methods are known that can be used for the definition of lignins on the basis of the production of specific degradation products, such as acidolysis and thioacidolysis. This type of definition (or other definitions based on properties) makes it possible to recognize protolignins, but it suffers from the drawback that it provides only limited information about their structures. Sarkanen and Ludwig (1971) proposed a definition of protolignins that is based on their biosynthesis from *p*-hydroxycinnamyl alcohols. Such a definition has some advantages and stresses some key features of protolignins. However, it suffers from the drawback that is does not *directly* provide characteristics that could be used for the discrimination between protolignins and other materials.

Considering what is required in connection with analytical work, it is useful to give a description of protolignins of the type described below. This description is not a chemical definition of protolignins, but summarizes the main structural features based on knowledge available today.

Protolignins are biopolymers consisting of phenylpropane units with an oxygen atom in the *p*-position (as OH or O–C) and with none, one or two methoxyl groups in the *o*-positions to this oxygen atom. These *o*-positions may

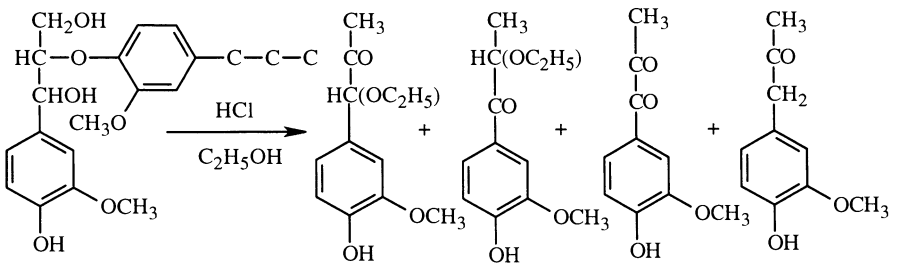
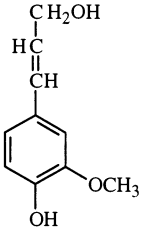


Fig. 4.1. Formation of Hibbert ketones from structural elements in lignin of the guaiacylglycerol β-aryl ether type on refluxing with HCl in ethanol

alternatively be *C*-substituted or *O*-substituted with other substituents than methoxyl (formulas **1–3**). Only a few of the aromatic units are substituted in other ring positions. A few percent of the building blocks in protolignins are not phenylpropane units. The side chain is missing or shortened, or the unit is replaced by a quinoid group. The phenylpropane units are attached to one another by a series of characteristic linkages (β-*O*-4, β-5, β-β, etc., see below) or, alternatively, exist as members of a series of characteristic end groups (e.g., cinnamaldehyde units, see below). Practically all the types of structural elements detected in protolignins have been demonstrated to be formed on oxidation of the *p*-hydroxycinnamyl alcohols **4–6** in vitro (Freudenberg 1968; Adler 1977). The structural elements in protolignins are not linked to one another in any particular order. Protolignins are not optically active. The polymer is branched and cross-linking occurs. In addition, the following facts should be noted: (1) there are strong indications of the occurrence of linkages between protolignin and carbohydrates, (2) some types of protolignins are esterified with phenolic acids (grass lignins with *p*-coumaric acid and certain other lignins, such as aspen lignin, with *p*-hydroxybenzoic acid), and (3) scattered observations suggest that there are some units, for example dihydroconiferyl alcohol units, that cannot be thought to have been produced on oxidation of *p*-hydroxycinnamyl alcohols.



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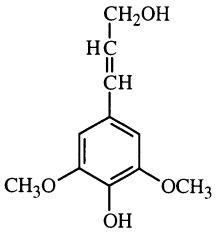

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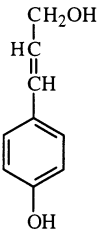


6

Protolignins as well as lignin after pulping and bleaching are studied in situ or as isolated samples. It should be kept in mind that an isolated lignin sample is not chemically, but operationally defined, i.e., it is defined by the starting material and the operations applied to it in order to obtain the sample. This implies that lignin preparations isolated from botanically and morphologically equivalent wood samples using different methods may have different properties (such as number of phenolic groups and molecular weight distribution). It also means that other materials than lignins may be present in the samples. Examination of the specimens may be required to verify their lignin nature and homogeneity. As a concrete example of this type of ambiguity it could be mentioned that proteins and tannins both give rise to so-called Klason lignin (TAPPI test method T-222 om-88). Furthermore, attention has to be paid to the possibility that chemical modifications have occurred during the isolation procedures.

Some of the drawbacks faced in connection with the use of isolated lignin samples can be avoided by studying the lignin in situ. In this case other constituents in the samples may interfere. Furthermore, wood and pulps are insoluble in unchanged form and this excludes the use of quite a few of the most powerful analytical techniques. In some cases, the biological microstructure is an obstacle when lignins are studied in wood or pulps. In many such studies of lignins, scientists have used property-based lignin definitions, including materials exhibiting particular color reactions, materials giving UV absorption at specific wavelengths, and materials giving rise to certain NMR signals. This is adequate in some contexts but may, in other cases, lead to erroneous results. In most cases, characterization of lignin requires studies of lignins in situ as well as of isolated lignin samples.

4.2 Structure, Nomenclature

4.2.1 Protolignin

The different types of phenylpropane units in lignins are denoted guaiacylpropane (**1**), syringylpropane (**2**), and *p*-hydroxyphenylpropane (**3**) (Sect. 4.1) The designation of the atoms in the phenylpropane skeleton is shown in Fig. 4.2. A variety of lignin units with different types of side chains (the units are framed in the figure) is shown in Fig. 4.3. For simplicity all these units are depicted as guaiacyl units. In some protolignins, 5–10% of the units are esterified with *p*-hydroxybenzoic acid (e.g., aspen lignin) or with *p*-coumaric acid (grass lignins). The units attached to **19** and **21** exemplify esterified units. The approximate distribution of units with different types of side chains in protolignins is given in Table 4.1. The distribution of certain types of lignin units in which the aromatic ring is connected to an adjacent unit is shown in Table 4.2.

4.2.2 Forms of Lignins after Pulping and Bleaching

During pulping, lignin undergoes more or less drastic degradation reactions depending on the pulping conditions. The composition of the lignin dissolved in pulping liquors as well as the composition of the lignin residue remaining in the pulp thus markedly differ from that of the native lignin. In the bleaching stages the residual lignin is successively degraded and dissolved and at most some of its structural features resemble those of the native lignin.

There is some confusion in the terminology. The terms "kraft lignin," "alkali lignin," and "sulfite lignin" are common, but these terms usually refer to the

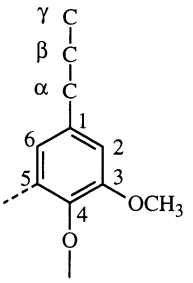


Fig. 4.2. Designations of the atoms in guaiacylpropane (**1**) and syringylpropane (**2**) units. In *p*-hydroxyphenylpropane units (**3**) the order of the numbering of the ring atoms is dependent on the substituents

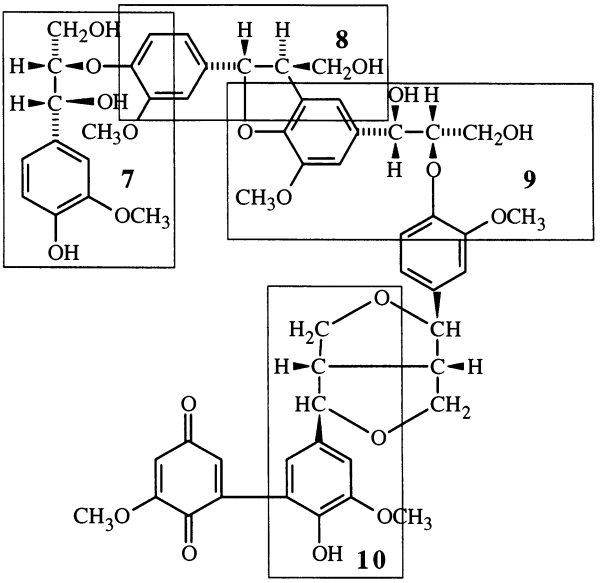
Table 4.1. The distribution of lignin units with different types of side chains (%; for numbering, see Fig. 4.3)[a]

Type of unit	Softwood lignin[b]	Hardwood lignin[c]
Units of the arylglycerol β-aryl ether type [**7** (*erythro*), **9** (*threo*)]	35	45
β-5 Units (**8**)	9	4
β-β Units (**10**)	3–4	7
β-*O*-4/α-*O*-4 Units (**11, 18**)	8	4
β-1 Units (**12**)	3	5
Units with arylconjucated carbonyl groups (**15, 17, 19**)	9	5
Glyceraldehyde 2-aryl ethers (**14**)	1–2	2
Cinnamyl alcohol units (**13**)	1–2	1–2
Arylglycerol units (**22**)	2	3
Units with carboxyl groups (**20, 21**)	4	4
Units with "reduced" side chain (e.g., **16**)	2	1
Quinone units (some of them lacking side chain, e.g., the *p*-quinone unit attached to **10**)	3	3
Units linked to *C*-6 or *C*-2 (e.g., **23**)	3	3
Dienone units (e.g., **25**)	2	2
Units of type **24**	2	2
Units attached to each other by α-β linkages	?	?

[a] Most of the data were obtained from studies of isolated lignin samples.
[b] Guaiacyl units strongly predominate.
[c] A hardwood lignin consisting of about equal amounts of guaiacyl and syringyl units is considered.

(soluble) lignin degradation products in spent liquors after pulping. Their composition markedly differs from the composition of the respective residual pulp lignins.

The main emphasis of this chapter is focused on the characterization of solid lignin samples. Even if lignin can be studied in situ, in most cases a pre-



p-quinone unit

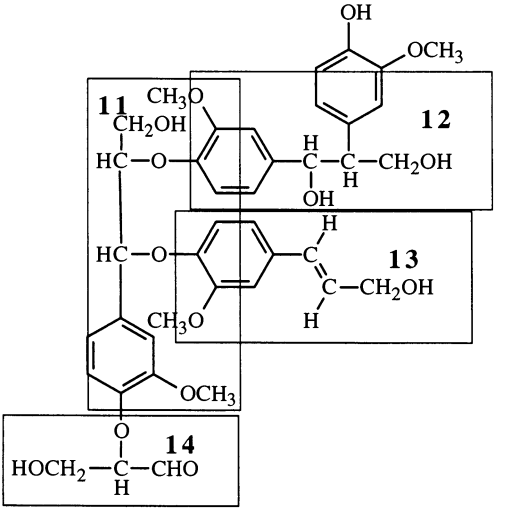
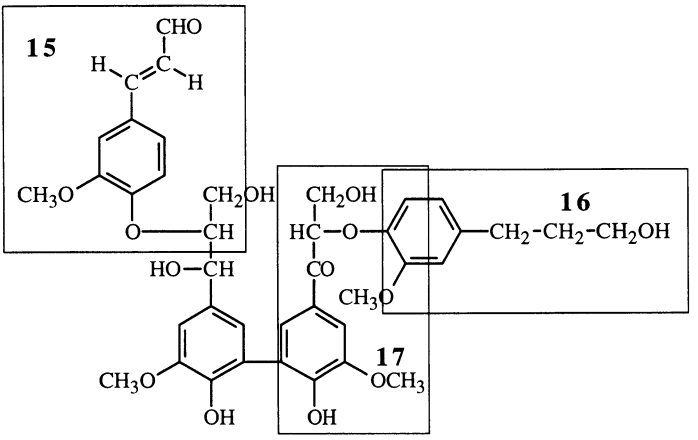


Fig. 4.3. Units (7-25) present in protolignin (for simplicity, all the units are depicted as guaiacyl units)



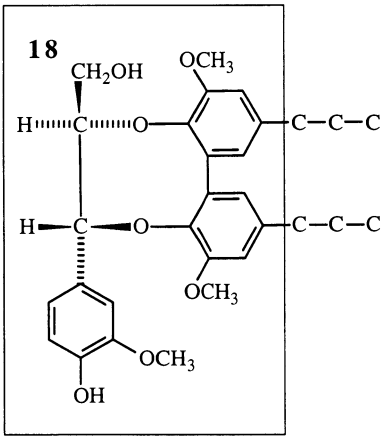


Fig. 4.3 *continued*

Table 4.2. The distribution of certain types of lignin units in which the aromatic ring is connected to an adjacent unit ("condensed units"; %; for numbering, see Fig. 4.3)

Type of unit	Softwood lignin	Hardwood lignin
Biphenyl units (e.g., the units linked to **18**, see also unit **10**)	20–25	10
Diaryl ether units (e.g., the unit linked to **19**)	4	7
β-5 Units (**8**)	9	4
Units connected to adjacent units via *C*-2 or *C*-6 (e.g., the unit attached to **23**)	3	3

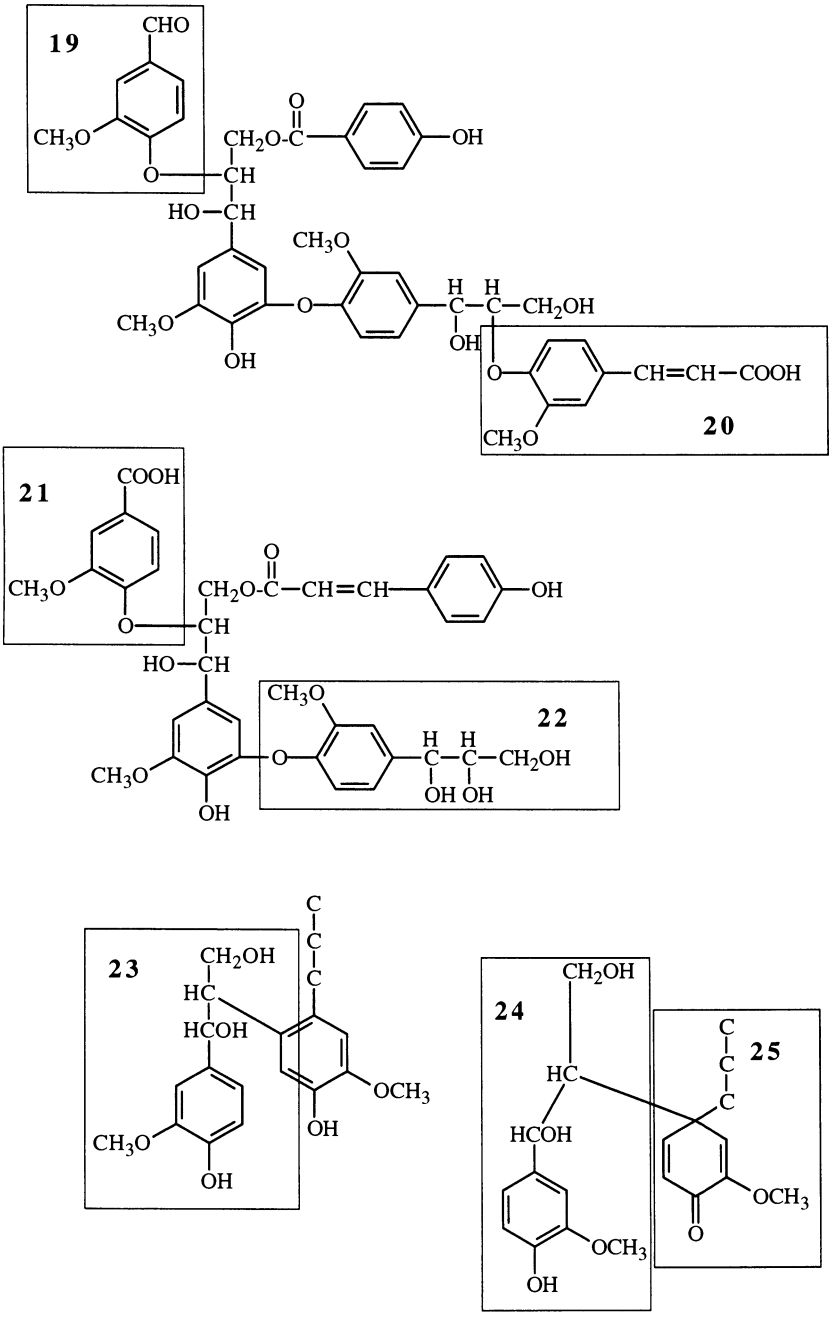


Fig. 4.3 *Continued*

vious isolation of it from the sample (wood or pulp) is necessary before the analysis. The final analytical methods used for the native wood lignin can usually be applied to the residual pulp lignin even if a more specific isolation technique is often applied.

For characterization of lignin degradation products in pulping liquors, bleach liquors, and process waters and effluents from papermaking, see Chapters 7, 8, and 9, respectively.

4.3 Detection and Classification

For a rapid answer to the question "Does this material contain lignin?", specific color reactions can be used to detect the presence (or absence) of lignin. For the detection to be unambiguous, more thorough studies have to be carried out. In this section, some methods are described that provide conclusive evidence for the presence of lignin in a sample. The procedures selected are comparatively simple and do not require access to sophisticated instruments.

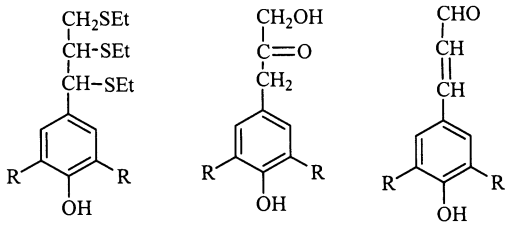
The best documented criteria for the classification of lignins according to structure is the degree of methoxyl substitution in the aromatic rings. The ratio of guaiacyl-, syringyl-, and *p*-hydroxyphenyl units (**1–3**; Sect. 4.1) is a convenient and often used basis for classification of a lignin sample. The accurate measurement of these ratios is difficult for a number of reasons, however, and each method has its limitations as will be discussed below. The study of the heterogeneity of lignin, i.e., the possible uneven distribution of types **1–3**, in the cell wall, is outside the scope of this description (see Chaps. 1 and 2).

In the following, the term *classification* is used for determination or estimation of the proportion of units of types **1–3** and for the contribution of phenolic acids attached to lignin by ester linkages (Sect. 4.2.1 and Fig. 4.3).

4.3.1 Detection of Lignin

Removal of low-molecular weight materials facilitates the detection of protolignin, since the interference of such products is avoided. Preliminary examinations may comprise color reactions (see below), and recording of the IR and UV spectra. Such preliminary experiments are to be complemented with examinations by degradation methods. Solvolytic methods, providing degradation products that must originate from protolignin, such as thioacidolysis (Sect. 4.6.1), acidolysis (Sect. 4.6.1), or ethanolysis (Sect. 4.1), are suitable for this purpose. The formation of the thioacidolysis products (**26**) or acidolysis

products (**27** and **28**) offer unambiguous evidence for the occurrence of protolignin in a sample.

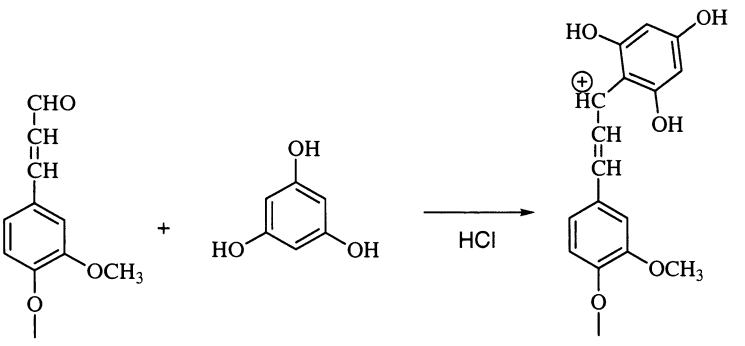


26 **27** **28**

R = H or OCH_3

For the detection of lignin products, solvolytic methods are still applicable as long as the chemically modified lignins still contain structures of the β-*O*-4 type (for examples, see Fig. 4.3, and Sect. 4.6). The detection of lignin in samples that are extensively modified requires other methods, e.g., permanganate (Sect. 4.6.1), or nitrobenzene oxidation (see below), or pyrolysis (see below and Kleen and Gellerstedt 1991).

Color reactions of lignins are mainly used in connection with studies of lignin in situ. Many color reactions are based on the occurrence of cinnamaldehyde groups (**15**, Fig. 4.3; Adler and Marton 1959; Nakano and Meshitsuka 1992). Such groups are present in protolignin and react with aromatic amines like *p*-aminobenzoic acid or certain phenols with formation of colored structures. Cinnamaldehyde groups give a purple color with phloroglucinol-hydrochloric acid, due to an acid catalyzed condensation to a colored adduct (the Wiesner reaction; Fig. 4.4).



λ_{max} = 550 nm

Fig. 4.4. Reaction of coniferaldehyde units with phloroglucinol/HCl (the Wiesner reaction)

Other color reactions that are based on reactions with functionalities typical of the side chains in lignins include the specific color reaction of coniferyl alcohol groups (**13**, Fig. 4.3) with *p*-nitrosodimethylaniline (Fig. 4.5; Lindgren and Mikawa 1957) and the reaction between quinone monochlorimide (*N*-chloro-1,4-benzoquinone monoimine) and benzyl alcohol groups (Fig. 4.6; Gierer 1956). The latter reaction proceeds with displacement of the side chain to form the blue indophenol chromophore, whereas the former reaction involves the γ-carbon of the cinnamyl alcohol group.

There are several specific color reactions based on the occurrence of phenolic groups of various types. Most of these tests are of little use for the detection of lignin in the presence of other phenolic materials such as tannins. Examples of tests based on inorganic reagents, that are frequently used for the detection of lignin in plant tissues, are the Mäule test (sequential treatment with potassium permanganate, hydrochloric acid, and ammonia) and the Cross and Bevan test (treatment with chlorine and aqueous sodium sulfite). The Mäule test gives a purple-red color with hardwoods while for softwoods the color is brownish. The chemistry behind the reaction is somewhat obscure (Nakano and Meshitsuka 1992).

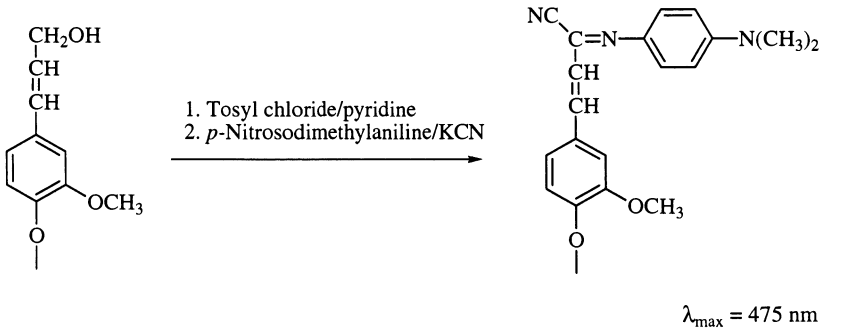


Fig. 4.5. Color reaction of coniferyl alcohol end groups

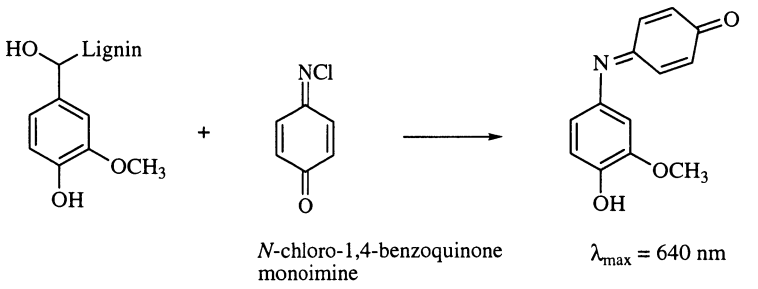


Fig. 4.6. Color reaction of benzyl alcohol groups

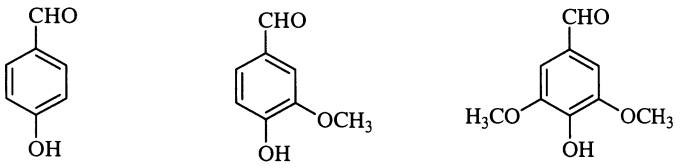
4.3.2 Classification of Lignins

Chemical Degradation. The above-mentioned solvolytic detection methods can also be used for the classification of lignins containing β-*O*-4 structures (protolignin and slightly modified protolignin). To classify strongly modified lignins, degradation methods that reveal the substitution pattern on the aromatic rings can be used. Permanganate oxidation is such a method (Sect. 4.6.1). Less elaborate degradation methods of this type are nitrobenzene oxidation and acid-catalyzed release of aromatic rings by nucleus exchange (see below). Pyrolysis is a facile procedure for the classification of lignin (see below).

The reaction of lignin with alkaline nitrobenzene results in the formation of aromatic aldehydes (Fig. 4.7; Leopold and Malmström 1952; Chen 1992a) and, to a small extent, the corresponding aromatic acids. The yield of degradation products is high. The nitrobenzene oxidation method has been extensively used for lignin classification (Chan et al. 1995a). Cupric oxide oxidation in alkaline solution is an alternative to nitrobenzene oxidation (Pepper et al. 1967; Chen 1992a).

The method referred to as nucleus exchange is based on the reversible formation of diphenylmethane structures when a lignin sample containing benzyl alcohol groups is treated with excess phenol and boron trifluoride (Funaoka et al. 1992). By using a sufficiently large excess of phenol, it is possible to release all the original aromatic nuclei from noncondensed units in both softwood and hardwood lignins (Fig. 4.8). For example, from a hardwood sample, a mixture of guaiacol and syringol is obtained together with their demethylation products. The method is not entirely specific for non-condensed lignin structures and some condensed units may also react to a certain extent (Chan et al. 1995b).

On rapid heating to temperatures around 500–600 °C in the absence of oxygen (pyrolysis), lignin is partly degraded to a complex mixture of low-molecular weight phenols (Meier and Faix 1992). Polysaccharides are degraded as well and a number of sugar-derived pyrolysis products are produced. This degradation method is rapid and requires only small amounts of



p-hydroxybenzaldehyde vanillin syringaldehyde

Fig. 4.7. Aldehydes produced on nitrobenzene oxidation of lignin

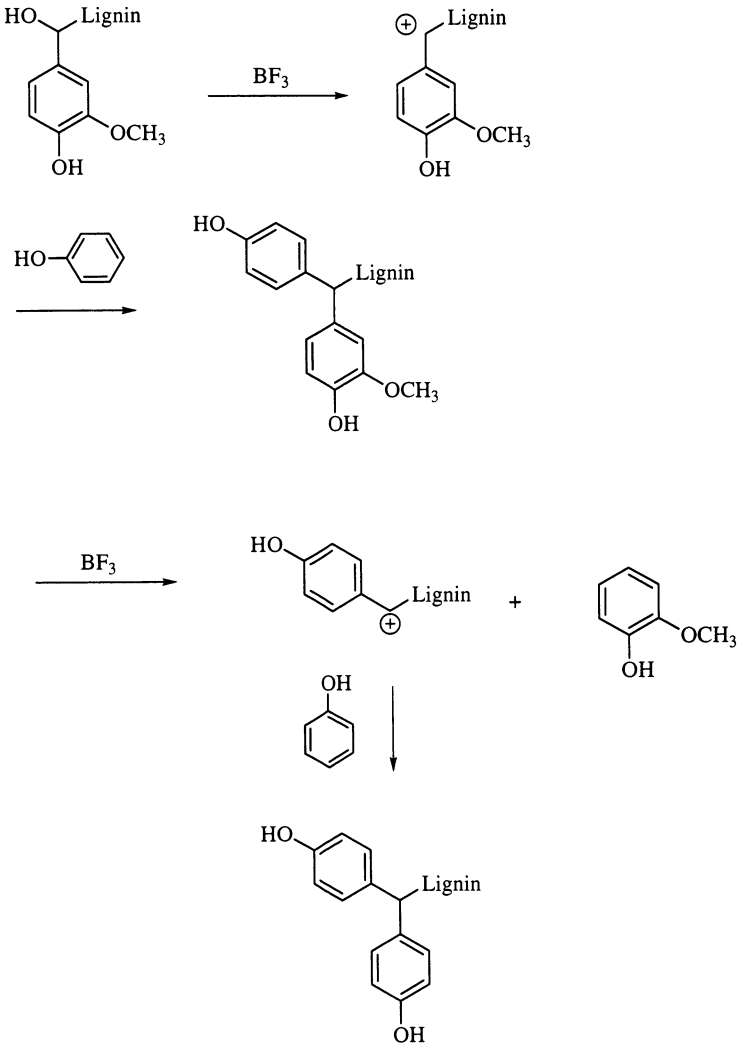


Fig. 4.8. An example of the nucleus exchange reaction. The liberation of guaiacol

sample (1–100 μg). The pyrolysis products can be analyzed by gas chromatography or gas chromatography/mass spectrometry. More than 50 pyrolysis products originating from *p*-hydroxyphenyl, guaiacyl, and syringyl units in lignins have been identified. The total yield of pyrolysis products is, however, rather low. The product pattern derived from the chromatogram (pyrogram) provides a basis for the classification of lignins.

Spectroscopic Methods. Spectroscopic methods used for structural elucidation (UV, IR, and NMR) can also be used for the classification of lignins. Electron

and UV microscopy are useful for the classification of lignins in intact plant materials and in pulp fibers. UV and NMR spectroscopy are treated in Section 4.6.2. IR spectroscopy is a valuable tool for the classification of lignin (Faix 1992). Of particular interest is the application of IR spectroscopy for the examination of solid samples. It is possible to estimate the ratio guaiacyl units (**1**)/syringyl units (**2**) (Sect. 4.1) by IR spectroscopic methods.

4.4 Quantitative Analysis

As already reviewed in Chapter 1, the content and composition of lignin varies considerably, not only among the wood species, but also in different morphological parts within the same species. At the conditions typical of mechanical and chemimechanical pulping, the lignin undergoes only moderate changes. After chemical pulping, however, marked structural changes take place both in the soluble lignin fraction and in the solid lignin residue (residual lignin) remaining in the pulp fibers. During the final (delignifying) bleaching, the lignin structure is extensively destroyed (Chap. 8).

4.4.1 Wood and Pulps

The oldest and most common method for the quantitative determination of lignin in wood as well as in mechanical, semimechanical, and semichemical pulps is based on gravimetry. In the *Klason lignin* determination (Standard of Technical Association of the Pulp and Paper Industry 1983; Dence 1992a), the sample is first treated with 72% sulfuric acid and subsequently heated with dilute acid to hydrolyze the polysaccharides to soluble fragments, mainly monosaccharides, after which the solid residue (Klason lignin) is washed, collected, dried, and weighed. The method can easily be adapted for use on a micro scale.

While undoubtedly the best procedure for determining lignin contents, provided standard conditions are strictly applied (Dence 1992a), the Klason method nevertheless has serious limitations that cannot be ignored. It gives correct values for the protolignin content of all softwoods. Hardwoods, however, contain variable amounts of so-called "acid-soluble lignin," a lignin that remains in the solution when the 72% sulfuric acid is diluted with water to 4–5%. Older values in the literature for the protolignin content of hardwoods are for this reason consistently too low. On the other hand, the kino material present in some hardwoods, such as eucalypts, causes their protolignin content to be overestimated by the Klason method.

The Klason method is not applicable to herbaceous plants, many of which have found widespread use in the pulping industry. Such plants, especially

when immature, contain variable amounts of protein, which will condense with the lignin when treated with sulfuric acid (Phillips and Goss 1935; Phillips et al. 1939). When inner bark is treated with sulfuric acid, not only its true protolignin but also the phenolic tannins, sometimes present in amounts exceeding that of the protolignin, will appear as Klason lignin.

It has been reported that softwood kraft pulps can be analyzed for their lignin content by the Klason method, but much of the lignosulfonates present in sulfite pulps is acid-soluble. (Lorås and Löschbrandt 1961) Pulps that have been bleached with lignin-removing agents such as chlorine, chlorine dioxide, oxygen-alkali, or ozone are not amenable to lignin analysis by the Klason procedure.

The acid-soluble lignin present in the liquor after the solid Klason lignin has been removed, can be estimated by UV spectrophotometry. Here too, however, difficulties are encountered. The sulfuric acid often converts some of the polysaccharide material to furfural and hydroxymethylfurfural, both of which absorb in the UV range. Their contribution may vary with the sample used, although it is assumed to be relatively small at 200–205 nm, corresponding to the wavelength at which lignin has its absorption maximum. The second lignin absorbance maximum at 280 nm is much weaker.

The UV absorptivity is approximately the same for all softwood lignins because of their similar structure. Unfortunately, this is not the case for the hardwoods, where the structure of the protolignin can vary from one tree species to another. The absorptivity of hardwood lignin must therefore be measured with authentic MWL or CEL (Sect. 4.5.1) samples. The same, of course, applies to the residual lignin products present in unbleached or bleached pulps.

For chemical and semichemical pulps, including pulps that have been subjected to bleaching, the most commonly employed method for lignin determination is the *kappa number*. This is an indirect method in which the pulp sample is oxidized with an excess of potassium permanganate under specified conditions (Dence 1992a). The kappa number is calculated from the consumption of permanganate. Other oxidizable structures in wood do contribute to the kappa number but a linear relationship has been found to exist between the Klason lignin content and the kappa number, at least for softwood kraft pulps.

The *chlorine number* determination is based on the reaction of chlorine with lignin that is more rapid than the reaction with carbohydrates. In the modified chlorine number determination, the pulp is reacted with acidified sodium or calcium hypochlorite and the chlorine consumption is measured titrimetrically. In contrast to the permanganate method, the chlorine number determination is applicable to all types of pulps irrespective of species, pulping process, yield or degree of delignification (Dence 1992a).

A convenient method of quantifying lignin in small samples (mg scale) is the *acetyl bromide method*. A wood or pulp sample is treated with acetyl bromide in acetic acid in the presence of perchloric acid, resulting in the com-

plete dissolution of the sample (Iiyama and Wallis 1988). The lignin content is determined by UV measurement at 280 nm. The values obtained are in fair agreement with lignin analyses by the Klason method. Even simpler is the method in which the sample is directly *dissolved in Cadoxen* (Sjöström and Enström 1966). However, the limitation of this method is the restricted solubility and it can be applied only to pulps with a low lignin content.

Direct quantification of lignin in wood and pulp by *near infrared spectroscopy* (NIR) can also be accomplished (Easty et al. 1990). This method requires a previous calibration based on, e.g., Klason lignin analysis of the same types of samples but once this has been done, a simple on-line detection can be arranged using fiber optics. The method is still in a development stage.

4.4.2 Aqueous Solution

Determination of lignin in solution is usually done by UV spectrophotometry, although quantitative measurement can be difficult due to lack of information on absorptivity values. For lignins dissolved in sulfite and kraft pulping processes, quantification of lignin at 280 or 205 nm is feasible after dilution since the aromatic structure is still intact. For very dilute solutions of lignin, fluorescence spectroscopy can be recommended, a technique that has a much higher sensitivity than UV measurements. It is possible to analyze natural waters for lignin material arising from pulp mills using fluorescence spectroscopy. Examples of fluorescence spectra are shown in Fig. 4.9 (Lundquist et al. 1978). A method with wide application is the so-called Pearl-Benson method (Nakano and Meshitsuka 1992). The reaction of phenolic units in lignin with acidified sodium nitrite leads to the formation of a nitrosophenol which, on addition of alkali, gives an intensely colored quinone mono-oxime. The absorbance is measured at 430 nm and is related to lignin by calibration with a standard lignin.

4.5 Isolation of Lignin

4.5.1 Wood

The close association between lignin and the polysaccharide constituents throughout the plant fiber wall makes it very difficult to isolate lignin completely from carbohydrate contaminants. In addition, the yield of isolated product is usually fairly low. The most commonly used method for the isolation of lignin involves extensive milling of the plant material followed by extraction with dioxane-water (Björkman 1956; Lundquist 1992a). The milling

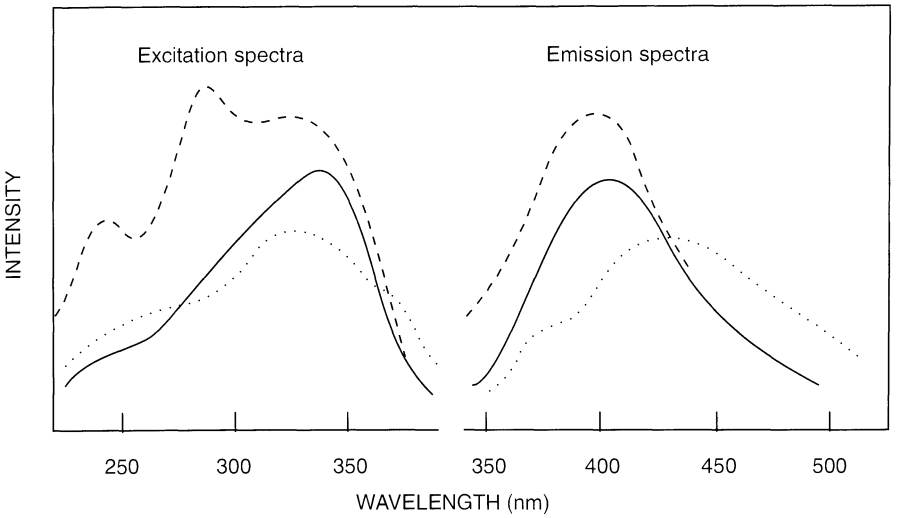


Fig. 4.9. Fluorescence spectra of water samples from the Baltic sea taken from surface and bottom regions. Lignin products and humic acids contribute to the fluorescence. Adapted from Lundquist et al. 1978

can be done either in a nonswelling medium like toluene or in the dry state. This lignin is usually referred to as milled wood lignin (MWL). The yields of lignin can be increased if the finely ground wood meal is treated with hydrolytic enzymes for removal of polysaccharides prior to solvent extraction. Such preparations are referred to as cellulolytic enzyme lignins (CEL; Chang et al. 1975). The further purification of MWL (Fig. 4.10), after removal of the solvent, can be done by precipitation in water from an acetic acid solution. Finally, the lignin is dissolved in 1,2-dichloroethane/ethanol and isolated by precipitation in diethyl ether. The yield is usually around 25%, based on the Klason lignin content.

MWL is considered to be representative of protolignin in wood and is widely used for structural studies of lignins in plants. The milling always causes extensive depolymerization and also some structural changes. It has been shown, for instance, that the number of phenolic groups tends to increase with longer milling times. There is also evidence of homolytic bond cleavage occurring during milling. It is therefore customary to keep the milling times relatively short, even if longer milling may give higher yields of lignin. The treatment with cellulolytic enzymes prior to the extraction with dioxane/water makes it possible to obtain CEL in amounts that correspond to a large percentage of the Klason lignin. The lignin prepared in this way contains comparatively large quantities of carbohydrates (probably lignin-carbohydrate complexes), although essentially carbohydrate-free lignin fractions can be prepared. An alternative method for the purification of MWL, based on liquid-liquid extraction is shown in Fig. 4.11 (Lundquist 1992a). This purification efficiently

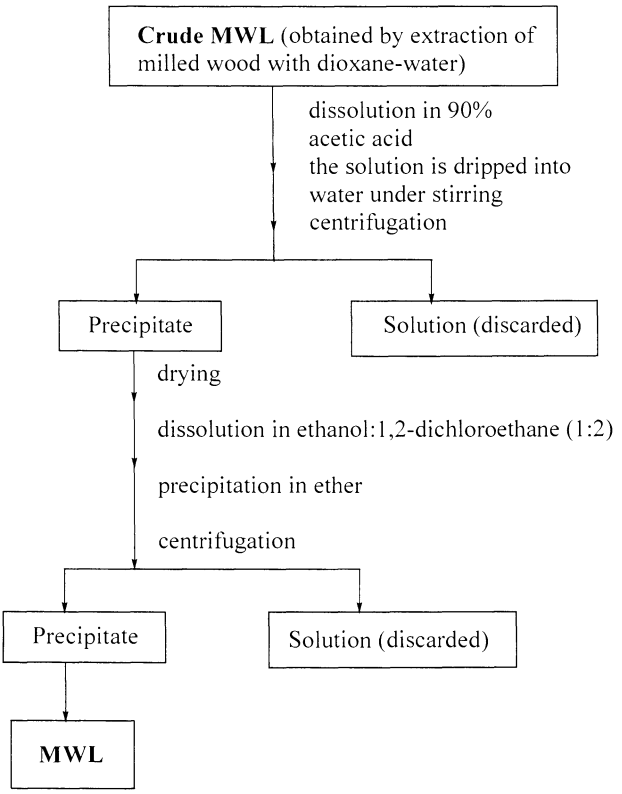


Fig. 4.10. Purification of MWL according to Björkman (1956)

removes carbohydrate contaminants. It is notable that MWLs from hardwoods contain larger amounts of carbohydrates (a few percent) than do MWLs from softwoods (traces). The molecular weight of hardwood MWLs is higher than that of softwood MWLs (Fig. 4.12; Lundquist et al. 1990).

4.5.2 Pulps

Isolation of lignin from chemical pulps is a difficult task because the lignin content is low and some of it is probably linked chemically to the carbohydrates. Two different methods have been employed for the isolation of lignin from chemical pulps, namely treatment with cellulolytic enzymes and acid hydrolysis. In the former case, a pulp sample is repeatedly treated with the enzyme mixture in such a way that virtually all polysaccharides are dissolved (Chang 1992). The method is similar to the cellulolytic enzyme method for the

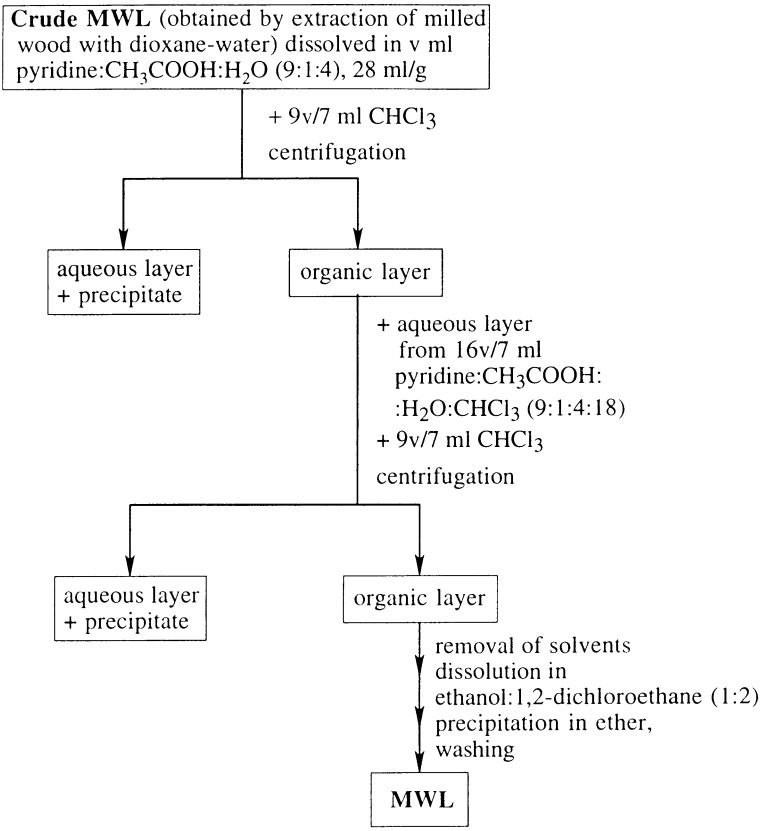


Fig. 4.11. Purification of lignin by liquid-liquid extraction

isolation of lignin from wood, the main difference being that chemical pulp, unlike wood, is digestible without milling. The residue is mainly composed of lignin but it is contaminated with carbohydrates and enzyme proteins. The latter can largely be removed by treatment with alkali followed by dialysis, leaving a lignin with approximately 0.6% (unbleached kraft pulp) nitrogen content. The lignin also contains some carbohydrates (3–7%) with a composition indicating both cellulose and hemicellulose origins. Possibly, because the carbohydrates are chemically linked to the lignin, neither prolonged enzyme treatment nor solvent extraction is effective for their removal.

The second method for the isolation of lignin from chemical pulps involves pre-extraction of the pulp with acetone for removal of extractives followed by an acid hydrolysis with hydrochloric acid in dioxane/water at reflux temperature (Gellerstedt et al. 1994; Fig. 4.13). This procedure liberates a carbohydrate-free lignin in good yield, which is isolated from the solution by evaporation of

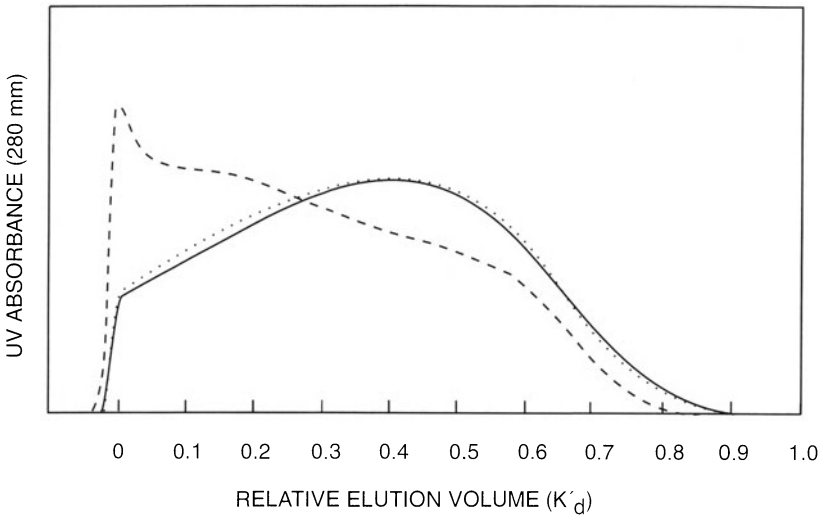


Fig. 4.12. Gel permeation chromatography of MWL from spruce (two samples, —— and .....) and birch (------). Sephadex LH 60/dimethylformamide-acetic acid. $K'_d = 0$ corresponds to the elution volume of a polymer excluded from the gel. $K'_d = 1$ corresponds to the elution volume of acetone. (Adapted from Lundquist et al. 1990)

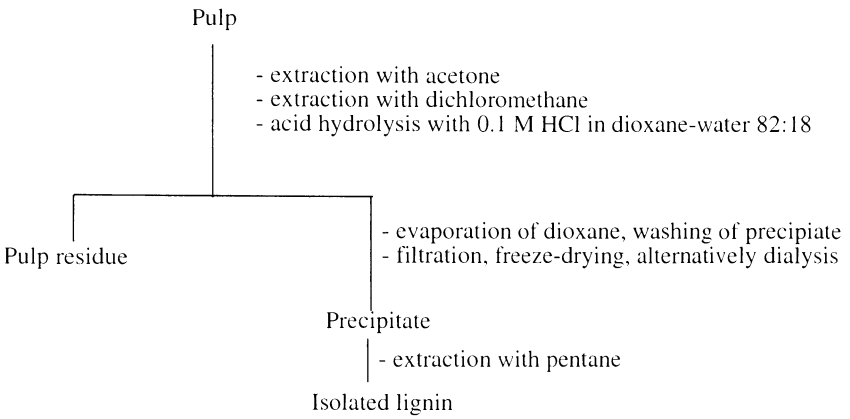


Fig. 4.13. Fractionation scheme for the isolation of residual lignin in pulp

the dioxane. The precipitated lignin is collected, washed free of acid, and further purified by continuous extraction with pentane.

This procedure can also be applied to partially bleached chemical pulps. The yield of isolated lignin, based on differences in kappa number, is, however, lower than for unbleached pulps because of higher water solubility of the

lignin. To some extent this can be circumvented by subjecting the aqueous lignin dispersion to dialysis rather than to precipitation, followed by freeze-drying.

Lignins subjected to acid hydrolysis may undergo chemical changes and it is well-known that cleavage of phenolic phenylpropane-β-aryl ether structures readily take place under such conditions (Sect. 4.6.1). In addition, condensation reactions, e.g., between aromatic *C*-6 positions and benzyl alcoholic groups in adjacent lignin side chains may occur. In kraft pulps, the residual lignin does not contain any appreciable numbers of β-aryl ether structures, however, and the number of benzyl alcohol groups is small. Therefore, the isolated lignins are assumed to be representative of the residual lignin although some chemical changes may have occurred during the isolation procedure.

4.5.3 Pulping Liquors

The techniques for the isolation of lignins from spent liquors from the pulping industry are based on the differences between lignin and other components in molecular weight or solubility (Lin 1992a). Neutralization of the phenolic hydroxyl groups in kraft lignin occurs in the pH range of 10–11, resulting in precipitation of the lignin. The acidification is usually done with carbon dioxide and sulfuric acid. Around 80% of the kraft lignin can be precipitated out from the black liquor with carbon dioxide under pressure at a pH of around 8. A further lowering of the pH with the addition of mineral acid results in an additional precipitation, and a total yield of lignin of about 90% can be obtained. At low pH values, the carboxyl groups in lignin are liberated (pK_a 3–5). The liquor is usually heated to improve the precipitation of the lignin. The isolated lignin is washed with acidic water to reduce the content of inorganic salts and organic contaminants. The kraft black liquor is a highly complex mixture, and the composition of the precipitated lignin depends on the conditions of precipitation. A lower methoxyl and a higher carboxyl content have been noted for lignins precipitated at lower pH values.

When lignin samples of high purity are required for analytical purposes, a neutralization of the kraft black liquor to a neutral pH is preferred prior to the isolation of the lignin. The isolated lignins still contain some impurities derived from extractives, carbohydrates, and sulfur. The impurities can be removed by continuous Soxhlet extraction with pentane followed by dissolution of the lignin in dioxane containing a small amount of water. Centrifugation of this solution removes carbohydrates and results in a lignin of high purity with only trace amounts of carbohydrates, which, presumably, are chemically linked to the lignin. Treatment according to the scheme in Fig. 4.11 provides an alternative method for the purification of lignin isolated from pulping liquors (Lundquist 1992a).

Step 1 Ion exchange

$$\text{Lignin-SO}_3\text{H} + \text{R}_4\text{N}^+\text{Cl}^- \longrightarrow \text{Lignin-SO}_3^-\ \text{R}_4\text{N}^+ + \text{HCl}$$

Step 2 Extraction with organic solvent

Step 3 Regeneration of lignosulfonate

$$\text{Lignin-SO}_3^-\ \text{R}_4\text{N}^+ + \text{NaOH (or other alkali)} \longrightarrow$$

$$\text{Lignin-SO}_3\text{Na} + \text{R}_4\text{N}^+\text{HO}^-$$

where R are hydrogens or alkyl groups

Fig. 4.14. The extraction of lignosulfonic acids as amine complexes

In acid sulfite pulping, lignosulfonic acids are formed. The sulfonic acid group is a strongly acidic group and it is dissociated at all pH values. Therefore, lignin cannot be isolated by adjusting the pH of the solution. However, one possibility is to precipitate the lignin by adding a cationic substance, e.g., a long-chain aliphatic amine, that forms a water-insoluble salt, soluble and extractable with an organic solvent (Lin 1992a). By this procedure, other components such as neutral sugars and inorganic ions are removed from the liquor. Subsequently, the amine-sulfonate complex is treated with aqueous alkali, which liberates the free amine in the organic solvent and the lignosulfonic acids can be recovered from the liquor as sodium salts. The reaction sequence is outlined in Fig. 4.14.

Membrane methods and ion exchangers can also be used for separation of kraft lignin or lignosulfonates from other constituents in pulping liquors (Lin 1992a,b).

4.6 Structural Analysis of Lignin

4.6.1 Methods Based on Degradation

Structural elucidation and analysis of chemical compounds are nowadays primarily accomplished by spectral methods. Chemical degradations and identification of the fragments obtained played an important role in such chemical work as recently as a few decades ago, and there is still a need for this approach in connection with the solution of some types of structural problems. Lignins have a very complex structure. To obtain a reasonably complete structural description of lignins (and several other biopolymers), both of the above-mentioned approaches are required in the structural analysis.

Four methods for the degradation of lignins are described below: acidolysis, thioacidolysis, permanganate oxidation, and ozonolysis. *Acidolysis* involves the degradation of the lignin by acid-catalyzed hydrolysis of ethers (primarily arylglycerol β-aryl ethers). Such ethers are also cleaved on *thioacidolysis*, but the splitting of the ethers is achieved by a Lewis acid (BF_3) catalyzed solvolysis (ethanethiol). To facilitate the analysis of dimeric thioacidolysis products, the thioacidolysis mixture is desulfurized by treatment with Raney nickel. *Permanganate oxidation* involves a preliminary methylation of phenolic groups and subsequent oxidation with permanganate. The oxidation converts the side chains to carboxylic groups, and a mixture of aromatic carboxylic acids is produced. *Ozonolysis* degrades the aromatic groups and a mixture of intact side chain fragments is obtained.

Nitrobenzene oxidation (Sect. 4.3.2) constitutes in certain respects an alternative to permanganate oxidation. *Hydrogenolysis* (catalytic hydrogenation; Sakakibara 1992) and *thioacetolysis* (Nimz 1974) are other important degradation methods. The composition of the reaction mixtures obtained by the last two methods resembles that resulting from the *thioacidolysis/Raney nickel* treatment. An interesting degradation method for the analysis of noncondensed and condensed lignin units is based on the so-called "nucleus exchange reaction" (Sect. 4.3.2). Recent model compound studies indicate, however, that this method leads to erroneous results since condensed units are partly converted into uncondensed units under the conditions prevailing during the treatment.

Acidolysis. The term acidolysis refers specifically to the refluxing (or heating at 100 °C) of the lignin sample with 0.2 M HCl in dioxane-water (9:1 v/v; Lundquist 1976, 1992b). This treatment was originally introduced in lignin chemistry as a procedure for isolating lignin from plant materials and as an analytical tool in lignin studies for determining the occurrence of β-*O*-4 structures and β-5 structures in spruce lignin. Acidolysis causes a selective cleavage of arylglycerol β-aryl ethers and some other types of labile ether linkages, and this has been utilized in several lignin studies. Treatment with the acidolysis reagent at 50 °C has been used for the selective hydrolysis of noncyclic benzyl aryl ethers. The acidolytic cleavage of ether bonds in lignin results in the formation of substantial amounts of low-molecular weight compounds (monomers and dimers). On the basis of comprehensive model compound studies, it has been possible to trace the degradation products to different types of lignin structures. Evidence has been obtained for the occurrence of structural elements in lignins of the β-*O*-4, β-5, β-β, β-1, glyceraldehyde-2-aryl ether, 2-aryloxypropiophenone, cinnamaldehyde, cinnamic acid, benzaldehyde, benzoic acid, and quinoid types. Acidolysis studies also offer possibilities for quantitative estimates of certain types of lignin structures. Analytical routine procedures have only been worked out for monomeric acidolysis products. They are based on gas chromatographic analysis of trimethylsilyl derivatives of the acidolysis monomers. Analysis of dimeric acidolysis products is,

however, also of value for the structural elucidation of lignins. The dimers can also be analyzed by gas chromatographic methods. The most important degradation reaction is the cleavage of β-*O*-4 structures (Fig. 4.15).

Acidolysis has the advantage that the degradation procedure is rather simple and requires only small amounts of samples. It is also noteworthy that several of the acidolysis products are structurally related to or identical to compounds formed from the lignin in wood during technical processes (Fig. 4.16). The composition of the monomer fraction obtained on acidolysis of lignin is rather complex, which complicates the analysis. Another drawback is that etherified syringylglycerol β-aryl ethers are very stable to acidolytic cleavage. This is of importance in studies of hardwood lignins since the proportion of syringyl units is large in such lignins. The stability of such β-ethers lowers

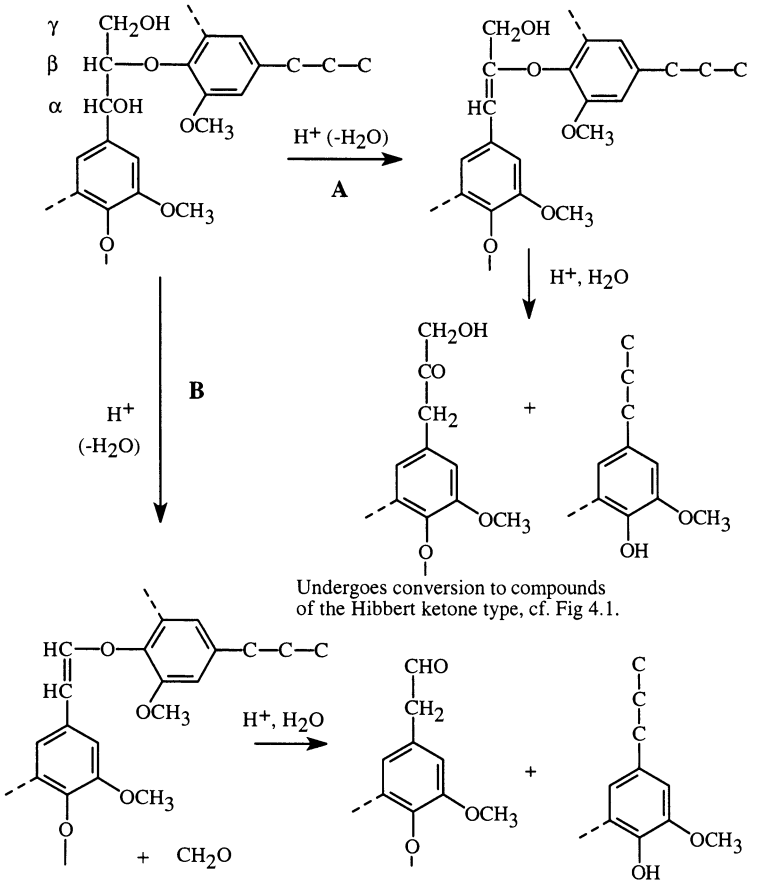
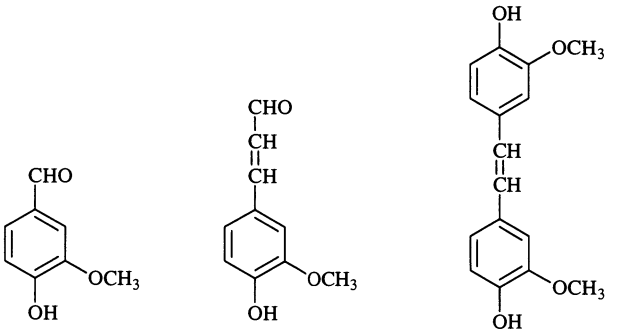


Fig. 4.15. Acid-catalyzed cleavage of arylglycerol β-aryl ethers occurs according to two reaction routes (**A** and **B**). Reaction route **A** predominates



Vanillin Coniferaldehyde Diguaiacylstilbene

Fig. 4.16. Examples of acidolysis products

the yield of acidolysis monomers of the syringyl type. Parenthetically, it can be mentioned that thioacidolysis (see below) does not suffer from these drawbacks. Acid-catalyzed condensation is of minor importance during acidolysis conditions.

The acidolysis procedure has recently been modified by replacing HCl with HBr (0.1 M) as the catalyst. HBr degrades lignin more efficiently than does HCl (Li et al. 1997). Furthermore HBr suppresses the side reaction leading to elimination of terminal hydroxymethyl groups as formaldehyde (reaction route **B** in Fig. 4.15).

Thioacidolysis. In thioacidolysis, lignin is treated with boron trifluoride and ethanethiol in dry dioxane, at 100 °C for 4 h (Rolando et al. 1992). This leads to extensive degradation, mainly by cleavage of arylglycerol β-aryl ether structures (see units **7** and **9** in Fig. 4.3). The mechanism of the reaction is shown in Fig. 4.17. Monomeric products are formed from noncondensed phenylpropane units. The mixture of monomeric products is silylated and analyzed by gas chromatography. The products are identified by mass spectrometry, and the yields determined from the peak areas in the gas chromatograms, using internal standards. The response factors are determined with the aid of products isolated in preparative experiments.

The structures of different monomeric products make it possible to detect the presence of *p*-hydroxyphenyl, guaiacyl, and syringyl units in a sample. Any change in the amount of arylglycerol β-aryl ether structures will be reflected in the yields of monomeric thioacidolysis products. Some minor products may also yield information about changes in the lignin structure. One of these, 1,1-di(ethylthio)-2-guaiacylethane, is found in small amounts in the thioacidolysis of softwood protolignin. In kraft lignins, the yields of this compound is much higher. This has been interpreted to indicate the presence of enol ethers

lacking the terminal side chain carbon (Fig. 4.18), a fact that is of interest in studies of the chemistry of kraft pulping.

Dimeric thioacidolysis products have a rather complex structure and comparatively high molecular weight. Desulfurization with Raney nickel converts

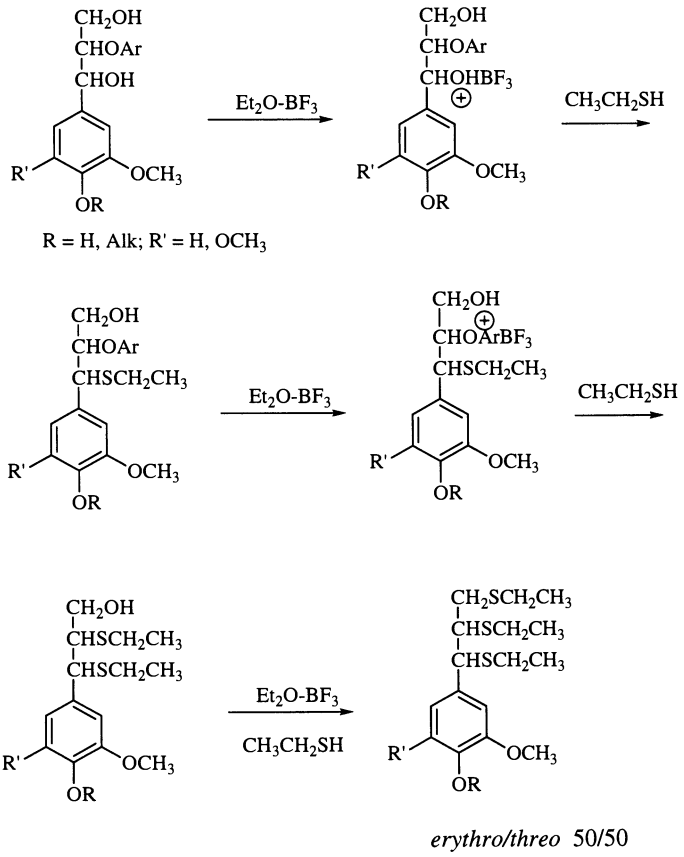


Fig. 4.17. Degradation of arylglycerol β-aryl ethers by thioacidolysis

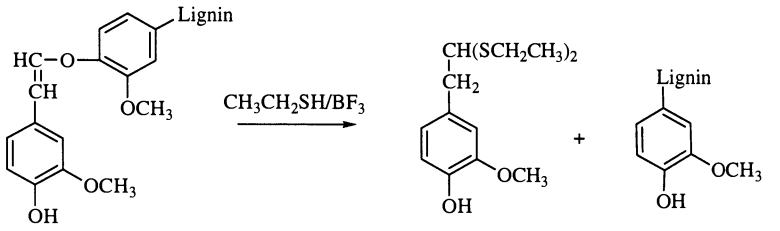
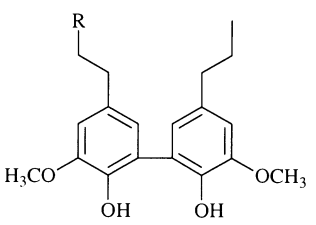


Fig. 4.18. The cleavage of enol ethers by thioacidolysis

the dimers to compounds with hydrocarbon side chains. These modified dimers can be readily analyzed by gas chromatography (Lapierre et al. 1991). From softwood lignins, five major types of dimers have been identified (Fig. 4.19). On the basis of model compound studies, the dimers have been assigned to the most important *C-C* bonded phenylpropane units (5-5, β-5, β-β, β-1) as well as 4-*O*-5 bonded structures. It is difficult to draw conclusions



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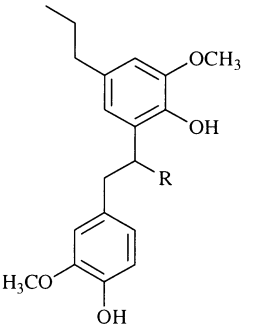

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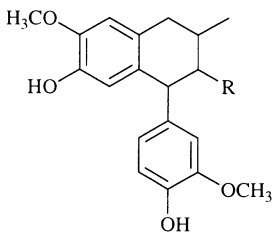

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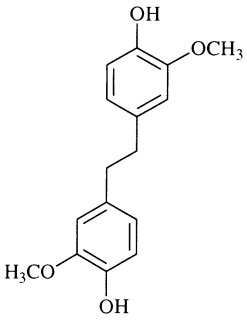


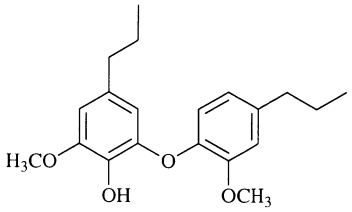


Fig. 4.19. Types of dimers formed after Raney nickel desulfurization of dimeric thioacidolysis products

concerning the frequencies of these structures in the lignin. Only the dimeric structures that are bound by hydrolyzable bonds to the rest of the polymer will appear among the thioacidolysis products. As a consequence, it is clear that thioacidolysis alone cannot give a complete description of the structure of lignin, but the yields and the ratios observed can be used for detecting changes in lignin structure caused by a given treatment such as pulping. The main advantage of thioacidolysis resides in its very efficient cleavage of β-ethers and in the small sample size necessary for analysis.

Permanganate Oxidation. The permanganate degradation is a two-step oxidative degradation. Prior to degradation with permanganate, the lignin is alkylated, usually with dimethyl or diethyl sulfate (Freudenberg 1968; Erickson et al. 1973; Gellerstedt 1992a). This alkylation protects the phenolic aromatic rings against oxidation. In the first step, alkaline permanganate degrades the side chains of the lignin, leaving the alkylated units as aromatic acids. Some α-keto acids are also formed which are converted to aromatic carboxylic acids in a second oxidation with hydrogen peroxide. The mixture of acids is esterified and analyzed by gas chromatography. The structures of the major degradation acids (**29–37**) are shown in Fig. 4.20. These structures provide information about the substitution pattern of the aromatic rings in the phenolic units of the lignin sample. The yields of the individual acids reflect the abundance of certain structural elements in the lignin. The monocarboxylic acids are formed from noncondensed phenolic groups, the dicarboxylic acids from units having an additional carbon substituent in the 5 or 6 position of the aromatic ring. The acids containing two aromatic rings reveal the presence of biphenyl and biphenyl ether structures. The presence of catechol units can be detected if the alkylation is carried out with diethyl sulfate. A large number of minor degradation products are also formed; together they represent only a few percent of the total degradation acids.

The permanganate degradation has been used for determination of the frequencies of the phenylpropane units corresponding to each acid (Erickson et al. 1973). This determination is based on the yields of the major degradation acids from MWL samples that have been treated with sodium hydroxide and cupric oxide prior to alkylation. This pretreatment liberates etherified phenolic hydroxyls and oxidizes aliphatic side chains to carbonyl groups, giving considerably larger yields of degradation acids (see Bose et al. 1998). Kraft pulping has a similar effect. The calculation of the frequencies of structural patterns is based on the assumption that the side chains are oxidized in similar yields, regardless of the type of structural unit they belong to. The actual yields of degradation acids are multiplied by a factor based on this assumed yield per carbon substituent, giving a "theoretical yield" for each acid. The theoretical yields are then converted to molar percentages by dividing each theoretical yield with the molecular weight of the methyl ester of the acid. These molar percentages are then reported as relative frequencies of substitution patterns in the lignin sample. The interpretation of such results implies several assump-

tions that become less valid when the method is applied to highly condensed lignin products, e.g., from pulping processes, which give low yields of degradation acids. Keeping this limitation in mind, structural information can still be obtained from such experiments. One example is that chlorination of aromatic rings during bleaching will cause the formation of chlorinated degradation acids. Another example is the formation of *C*-6 condensation products during acidic treatment (as in sulfite pulping) which causes an increase in the yields of metahemipinic acid **34**.

The fact that only unetherified phenolic units are detected by permanganate degradation has been used to estimate the amount of phenolic groups released on treatments such as kraft pulping. Assuming that such treatment mainly cleaves arylglycerol β-aryl ethers, it is possible to estimate the amount of such ethers in the sample.

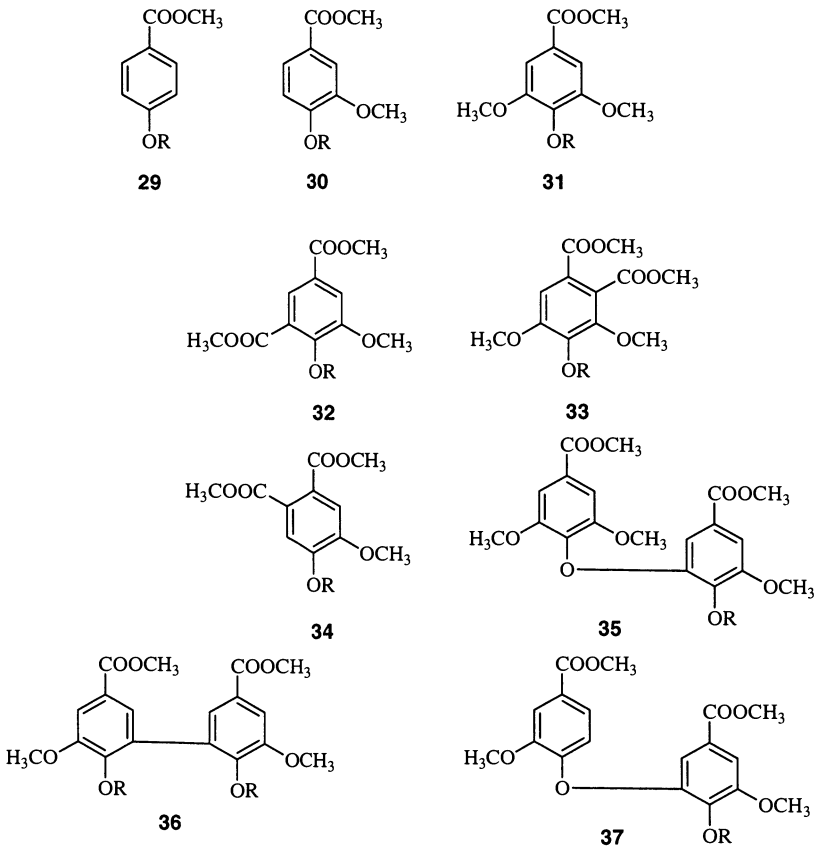


Fig. 4.20. Different types of aromatic acids formed on permanganate oxidation of lignin

The permanganate degradation is rather time-consuming and is difficult to apply to very small samples. But the method still gives information about lignin structure that is difficult to obtain otherwise, and is thus a useful complement to other methods.

Ozonolysis. Ozone attacks the double bonds in alkenes as well as aromatic rings. Hydrolysis or reduction of the initially formed ozonides leads to the liberation of cleavage products. Structural analysis of lignin by ozonolysis can be applied to isolated lignin samples as well as lignin in situ (Sarkanen et al. 1992).

Ozonolysis of lignin results in a degradation of the aromatic rings. In most cases the side chains remain intact. The aromatic rings originally attached to the side chains are replaced by carboxyl groups or, if they are connected to the side chain via ether linkages, by hydroxyl groups. A hydrolysis step is performed under alkaline conditions in order to hydrolyze ester groups. Ozonolysis of *erythro* forms of arylglycerol β-aryl ethers gives erythronic acid (Fig. 4.21). Analogously, threonic acid is produced from *threo* forms of arylglycerol β-aryl ethers (Fig. 4.21). The acids can be converted to trimethyl silyl derivatives and analyzed by gas chromatography. The number of arylglycerol β-aryl ether structures and the distribution of their diastereomeric forms in the lignin can be estimated on the basis of the results (Taneda et al. 1989).

Phenylcoumaran as well as 1,2-diaryl-1,3-propanediol structures on ozonolysis give rise to 2-hydroxy-3-hydroxymethylbutanedioic acid. Since only the *trans* form of phenylcoumaran structures is known to be present in lignins, such structures should merely give rise to the *erythro* form of 2-hydroxy-3-hydroxymethylbutanedioic acid (**38**; Fig. 4.22). Since both *erythro* and *threo* forms of 1,2-diaryl-1,3-propanediol structures may be present in lignins, a

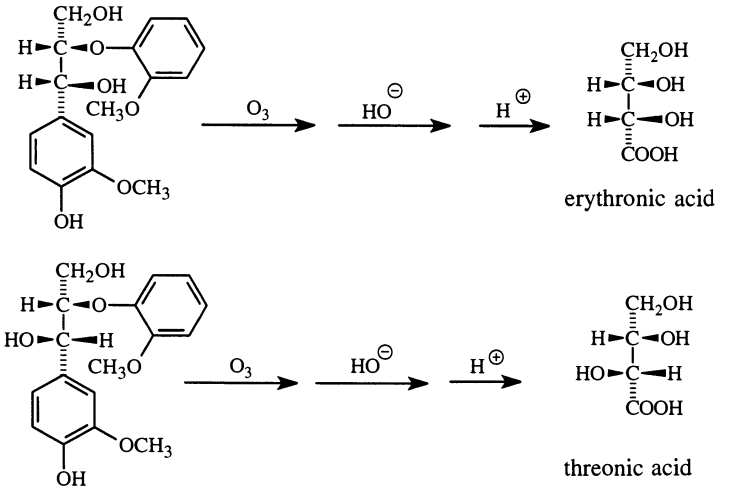
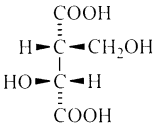


Fig. 4.21. Ozonolysis of β-ether model



39

mixture of the two diastereomeric forms of the acid can be expected to be produced from such structures. It follows that the amounts of acid **39** obtained on ozonolysis of a lignin sample together with the ratio of the diastereomeric forms, provide a basis for estimates of the proportions of these two types of structural elements in lignins (Habu et al. 1990).

Side chains with double bonds (as in cinnamyl alcohol, cinnamaldehyde, and cinnamic acid units) or side chains in other types of end groups have not been analyzed by ozonolysis.

Examples of the application of ozonolysis for the analysis of structural elements present in protolignins are given above. Residual pulp lignin can be analyzed for the same types of structural elements by the same methods. The results will show the changes of the proportions as well as of the distribution of diastereomers caused by the processing of the lignocellulosic starting material. Such changes influence the properties (e.g., location and intensity of NMR signals) and are therefore of practical interest from an analytical point of view. Residual pulp lignin can also be analyzed for structures not present in protolignin. Ozonolysis of Klason lignin gives, for example, rather large amounts of the acid **41** (Fig. 4.23; Matsumoto et al. 1986). This acid could originate from

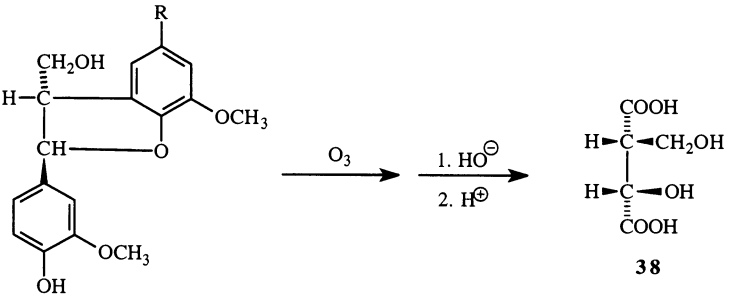
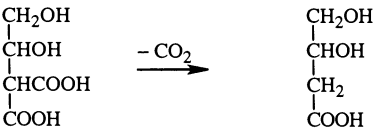


Fig. 4.22. Ozonolysis of phenylcoumaran (β-5) model



40 41

Fig. 4.23. Ozonolysis products from diphenylmethane structures

condensation products of arylglycerol β-aryl ethers (diphenylmethane structures). An initially formed malonic acid (**40**) is supposed to be decarboxylated during the ozonolysis to give **41** (Fig. 4.23).

4.6.2 Spectroscopic Methods

As mentioned previously, spectroscopic methods constitute important tools in connection with the analysis of lignins. A variety of spectroscopic techniques have been used for such purposes. In this section we focus on NMR (nuclear magnetic resonance) spectroscopic methods (primarily ^{1}H NMR and ^{13}C NMR) and also to some extent on UV (ultraviolet)/Vis (visible) spectroscopy.

The selection of suitable methods (or combination of methods) for the detection, characterization, and quantitative analysis of lignin in a sample is strongly dependent on the extent of information and the accuracy of the analyses required ccf. Sects. 4.3 and 4.4). In many cases, spectral methods other than NMR spectroscopy are satisfactory. Simplicity is also a factor that often determines the choice of methods. It is notable that even solid materials can be examined by NMR spectroscopy (cross polarization/magic angle spinning nuclear magnetic resonance, CP/MAS NMR; Leary and Newman 1992).

Quantitative Analysis of Functional Groups (see also Sect. 4.7). ^{1}H NMR spectral analysis of acetate derivatives (for a review see Lundquist 1992c) can be used for the determination of aliphatic hydroxyl (acetate signal), phenolic hydroxyl groups (excepting those in biphenyl structures, acetate signal), and benzyl alcohol groups (Hα signal). Total hydroxyl groups can analogously be determined from ^{1}H NMR spectra of propionate derivatives; this could be an advantage in the analysis of residual pulp lignin since the interference with signals from other groups can be expected to be low in the range where the propionate signals are located. ^{13}C NMR spectra of acetate derivatives (for a review of ^{13}NMR spectroscopy of lignin acetates and nonderivatized lignin see Robert 1992) can be used for the determination of total hydroxyl, primary hydroxyl, benzyl alcohol, and phenolic hydraxyl groups (Robert and Brunow 1984). The analyses are based on the signals from the carbonyl carbon in the acetate groups. These signals (centered at about δ 170) are located in a spectral region with very few other signals (Fig. 4.24). Methods for the analysis of different types of hydroxyl groups in lignins based on ^{31}P NMR spectroscopy have recently been developed (Argyropoulos 1995). ^{1}H NMR spectra of lignins in dimethyl sulfoxide solution allow the analysis of phenolic groups based on the hydroxyl signals (located in the spectral range δ 8–10; Fig. 4.25; Li and Lundquist 1994). UV spectroscopy (Δε for ionization; Aulin-Erdtman and Sandén 1968 and preceding work) has been used for a long time for the analysis of phenolic groups in lignins; the method has some weaknesses but is advantageous in some instances.

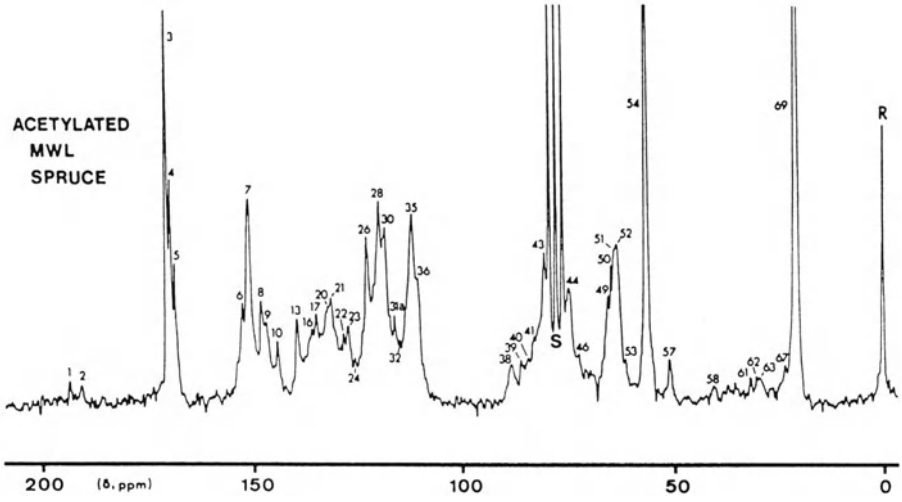


Fig. 4.24. ^{13}C NMR spectrum of acetylated spruce MWL

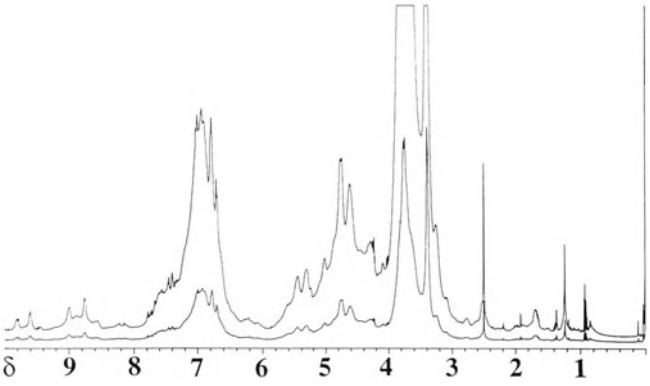


Fig. 4.25. ^{1}H NMR spectrum (500 MHz) of spruce MWL in dimethyl sulfoxide

Carboxyl groups can be analyzed by ^{13}C NMR as well as ^{1}H NMR spectroscopy. However, the signals are rather broad (particularly in the case of ^{1}H NMR), and interference with signals from other groups may occur. Methods based on ^{31}P NMR spectroscopy seem to be well suited for the analysis of carboxyl groups (Argyropoulos 1995).

Methoxyl groups can be analyzed by ^{13}C NMR spectroscopy (also in solid samples using CP/MAS). In ^{1}H NMR spectra there is an interference between the methoxyl signal and other signals. However, under certain conditions (acetate derivatives, pyridine solution) rough estimates of the methoxyl contents can be made.

Lignins can be analyzed for different types of carbonyl groups by UV and ^{1}H NMR spectroscopy of the lignin samples and derivatives of them obtained by borohydride reduction and acetylation; techniques based on difference spectra are applied (Lundquist 1992d). Carbonyl groups can also be analyzed by ^{13}C NMR spectroscopy but this technique has only been used to a limited extent for this purpose. A method for the analysis of carbonyl groups based on ^{19}F NMR spectroscopy (the carbonyl groups are converted into derivatives containing the trifluoromethyl group) has recently been developed (Ahvazi and Argyropoulos 1997). Certain types of carbonyl groups in quinoid structures can be analyzed by ^{31}P NMR spectroscopy after derivatization (Argyropoulos 1995).

Analysis of Different Types of Structural Elements (Aromatic Nucleus and Side Chain). Analyses of structural elements in lignins of types **7–25** (Fig. 4.3) are primarily based on spectral methods or studies of degradation products. NMR spectroscopy is the most important spectral method in such lignin analyses but UV/Vis spectroscopy is of great value for the determination of certain types of units.

Units **7** and **9** (Fig. 4.3) represent the two diastereomeric forms of arylglycerol β-aryl ethers – the quantitatively most important type of structural element in lignins. Proof of their existence and determination of their quantitative contribution can be achieved by ^{1}H NMR as well as ^{13}C NMR spectral methods. Units of type **8** (one of the units in the phenylcoumaran structure, **8–9**) have been analyzed by a UV spectroscopic method (after acid-catalyzed conversion of the phenylcoumaran structure into a phenylcoumarone; Adler and Lundquist 1963). The results have later been confirmed by NMR spectroscopic methods (^{1}H NMR, ^{13}C NMR) although some uncertainty regarding the quantitative contribution remains. Unit **10** is representative of phenylpropane units in the pinoresinol type of structural element. Such units (and the analogous syringaresinol structures) have successfully been analyzed by NMR spectral methods (^{1}H NMR, ^{13}C NMR). Units **10, 17,** and **18** represent phenylpropane units attached to an adjacent unit by a biphenylic linkage. Several such units are present in lignins. Their frequency has been estimated by UV and ^{13}C NMR spectroscopic methods. The occurrence of dibenzodioxocin **18** has recently been demonstrated using 2D NMR spectroscopic methods (Karhunen et al. 1995).

Attempts to detect units of type **11** (representing noncyclic benzyl aryl ethers, Fig. 4.3) by 1-D NMR spectral methods have failed and recent 2-D and 3-D NMR spectral studies suggest that only trace amounts of such units are present in lignins (Fig. 4.26; Kilpeläinen et al. 1994a,b).

Estimations by different methods of the contribution of units of type **12** (Fig. 4.3, present in the β-1 type of structure) to the lignin structure lead to contradictory results: degradation studies indicate that a substantial number of such units are present in lignins, while NMR spectral studies suggest that their proportion is small. Recent 2-D NMR examinations confirm the existence

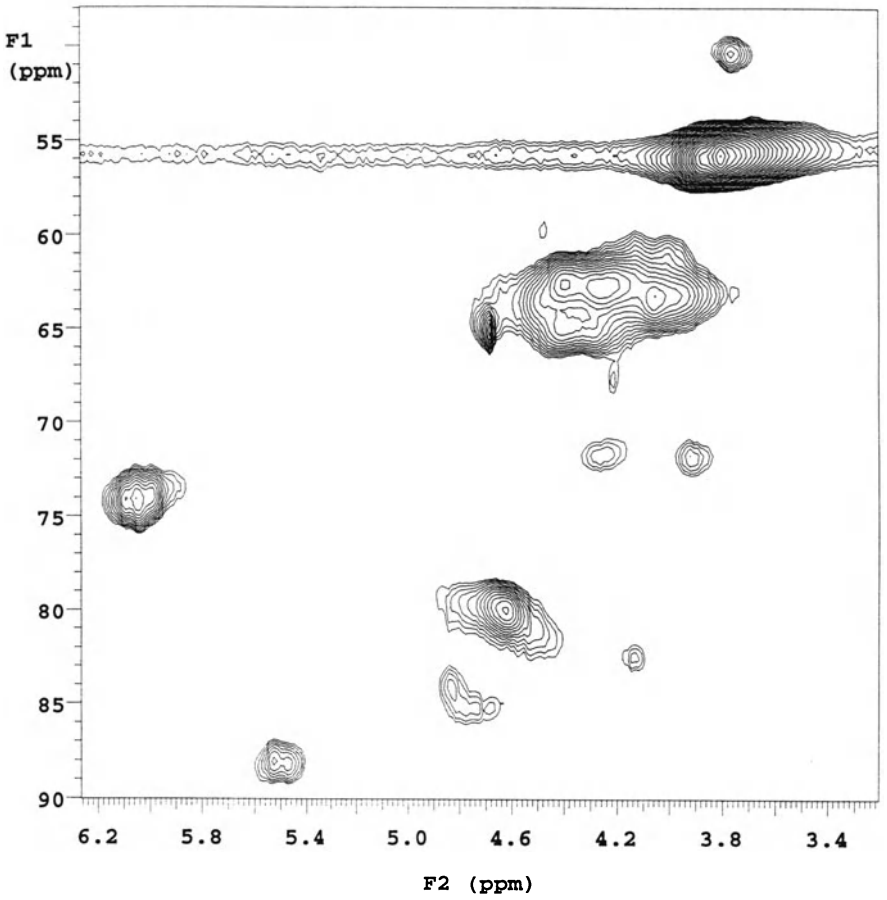


Fig. 4.26. HMQC spectrum of acetylated pine MWL

of units of type **12** in lignins (Kilpeläinen et al. 1994a), but the problems related to their frequency are not yet solved.

Only small amounts of coniferyl alcohol end groups (**13**; Fig. 4.3) are present in lignins. They can be analyzed by Vis spectroscopy after derivatization (Sect. 4.3.1). Their frequency in isolated lignin samples can be determined by ^{1}H NMR spectroscopy (Li and Lundquist 1994). Small amounts of detached side chains of type **14** are present in lignins; they can be analyzed by ^{1}H NMR spectroscopic methods after chemical modification, involving either reduction to glycerol derivatives or conversion into methyl acetals (Brunow and Lundquist 1991).

A few lignin units of type **15** (Fig. 4.3, coniferaldehyde end groups) are present in lignins. Such units can be analyzed by Vis spectroscopy after derivatization, for example by treatment with phloroglucinol/acid (the Wiesner reac-

tion, Sect. 4.3.1). Examination of isolated lignin samples by NMR spectral methods provides proof of their existence and it is also possible to determine their amount by using ^{1}H NMR spectroscopy (formyl proton signal). 1-D NMR spectral studies indicate the presence of trace amounts of units of type **16** in lignins. Their existence has been confirmed in recent 2-D NMR spectral examinations.

Unit **17** (Fig. 4.3) represents the 1-aryl-2-aryloxy-1-propanone type of unit. The fact that it is linked to an adjacent unit by a biphenyl linkage is incidental. It has been suggested, on the basis of UV spectroscopic studies, that the proportion of such units is rather large (Adler and Marton 1959) but this is gainsaid by ^{1}H NMR spectral studies. The discrepancy can be explained by the fact that units of type **19** are included in the UV spectroscopic estimates. Evidence for the presence of syringyl analogs of units of type **17** in a hardwood lignin has recently been obtained in 2-D NMR spectral studies (Kilpeläinen et al. 1994a).

The occurrence of a small number of units of the benzaldehyde type (**19**; Fig. 4.3) in lignins has been demonstrated by NMR spectral methods; ^{1}H NMR (formyl proton signal) and ^{13}C NMR (carbonyl group signal). The detection of ferulic acid units (**20**) by ^{13}C NMR spectral methods has been reported (Ralph et al. 1997). Units of the benzoic acid type (**21**) exist in lignins and in particular in lignin products obtained by oxidative modification. NMR spectral methods can be used for the analysis of such units; signals from the CH-groups in the aromatic ring adjacent to the carboxyl-substituted carbon can be used in the analyses.

2-D NMR spectral examinations have revealed the presence of small amounts of arylglycerol units (**22**; Fig. 4.3) in lignins (Kilpeläinen et al. 1994a). Spectral evidence for units **23–25** has not been reported so far. Certain types of lignins are esterified with phenolic acids (see the formula sheets involving unit **19/20** and **21/22**); the occurrence of such acids in lignins can be studied by NMR spectral methods (in particular ^{13}C NMR). Important results have been obtained by ^{13}C NMR analyses of plant materials enriched in or labelled with ^{13}C (Lapierre et al. 1984; Terashima et al. 1991). The use of ^{13}C enriched samples is particularly important in 2-D and 3-D NMR. The combination of ^{13}C labelling and CP/MAS is also of great interest since it makes it possible to obtain structural information of lignins in situ.

In many cases, structural elements of the types present in protolignin are also found in lignin after pulping. However, their proportions can be expected to differ from those found in protolignin. Oxidative treatment of lignin may, for example, result in the conversion of various types of structural elements in the lignin moiety into units of types **19** and **21** (Fig. 4.3). Generally, the analytical approaches used for protolignin can also be applied for the analysis of various lignin samples in several instances. Pulping and bleaching of lignocellulosic materials also leads to modifications of the lignin portion, resulting in the formation of different types of structural elements that do not exist in the starting material, such as stilbene structures, enol ethers, catechol structures,

units carrying sulfonic acid groups, and products of oxidative ring opening. In several cases it is possible to develop NMR or UV/Vis spectroscopic methods for the analysis of these newly formed lignin structures.

4.7 Analysis of Functional Groups in Lignin (see also Sect. 4.6.2)

Methoxyl Groups. The methoxyl content is a widely used basis for characterization of lignins. It gives a measure of their purity and of their plant origin. The method most widely used is the one originally introduced by Zeisel and is based on the formation of methyl iodide when the sample is treated with hydriodic acid at reflux temperature (Chen 1992b). The methyl iodide formed is then treated with bromine which liberates the iodine and converts it to iodic acid. The iodic acid is treated with potassium iodide and the liberated iodine is titrated with standard thiosulfate solution. Methoxyl/aryl carbon atom ratios have also been measured by ^{13}C NMR (Obst and Landucci 1986).

Phenolic Hydroxyl Groups. The chemical and physical characteristics of lignin are to a large extent determined by the presence of phenolic hydroxyl groups, which have an important influence on its solubility and reactivity during pulping.

Common instrumental methods for the estimation of phenolic groups in lignin are potentiometric and conductometric titration, ionization difference UV spectroscopy, and NMR spectroscopy (Lai 1992). Chemical methods include determination of the increase in methoxyl content after reaction with diazomethane, selective deacetylation of acetylated lignin with pyrrolidine (aminolysis), and determination of the amount of methanol formed after oxidation with sodium periodate. A more indirect method is based on permanganate oxidation after alkylation of the phenolic groups. In the following, some commonly used methods are described.

The UV method is based on the difference spectra obtained when subtracting the UV spectrum of the sample in neutral solution from the spectrum obtained in alkaline solution, where the phenolic hydroxyl groups are ionized (Aulin-Erdtman and Sandén 1968 and preceding work). The measurements are carried out in the range of 250–350 nm; the method is used for soluble samples. It requires calibration using appropriate model compounds. The method has also been applied to lignin in wood and pulp samples.

The titration method is based on the acidity of phenolic hydroxyl groups. Because of their weaker acidity, phenolic hydroxyls can be titrated in the presence of carboxyl or sulfonate groups.

The periodate method is based on the oxidation of guaiacyl and syringyl structures with aqueous sodium periodate to *o*-quinone structures with the release of one mole of methanol from each phenolic unit (Fig. 4.27). The

amount of methanol released is measured by gas chromatography. No methanol is formed from etherified groups. The method cannot be applied to materials containing methoxyl-free structures such as catechols or *p*-hydroxyphenyl groups. The good reproducibility and comparative simplicity of the method makes it useful for routine purposes (Francis et al. 1991).

The aminolysis is based on the finding that phenolic acetates are cleaved with pyrrolidine much faster than aliphatic acetates. The pyrrolidine acetate formed in the reaction of an acetylated lignin is determined by gas chromatography (Fig. 4.28). The aminolysis is a rather time-consuming method. Interference from the reducing end groups of carbohydrates makes it necessary to reduce the sample with borohydride prior to the aminolysis.

In an acetylated lignin sample, the carbonyl carbon signals in a ^{13}C NMR spectrum allow differentiation of primary, benzylic, and phenolic acetates. A spectrum run under quantitative conditions will allow estimation of the amounts of these three functionalities. This and other NMR spectroscopic methods are described in Section 4.6.2.

Aliphatic Hydroxyl Groups. The total amount of hydroxyl groups in a lignin preparation can be determined by acetylation with acetic anhydride and pyri-

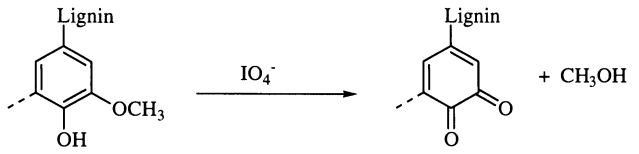


Fig. 4.27. Determination of phenolic groups by analysis of methanol liberated on oxidation with periodic acid

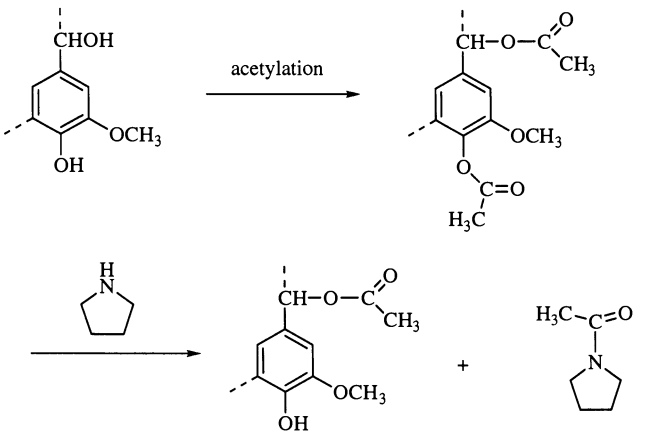


Fig. 4.28. Analysis of phenolic groups by aminolysis of acetate derivatives

dine followed by hydrolysis of the acetate and titration of the liberated acetic acid with a standard sodium hydroxide solution (Chen 1992c). The aliphatic hydroxyl content is determined by subtraction of the phenolic hydroxyl content (see above) from the total hydroxyl content of the lignin. In the case of lignins that contain carboxyl groups, direct titration of the liberated acetic acid will give erroneously high values for the hydroxyl content. In such cases, the liberated acetic acid must be either distilled off or be determined by gas chromatography. The presence of carbohydrates significantly affects the accuracy. It is therefore necessary to determine the carbohydrate content prior to performing hydroxyl group analysis.

Units Containing Double Bonds. Lignins can contain a number of different double bonded structures (Dence 1992b). In protolignins, these are mainly cinnamyl alcohol and cinnamaldehyde groups. Alkaline treatment gives rise to stilbene structures and styryl ethers. Treatment of lignin with acids produces stilbene and phenylcoumarone structures. Oxidative treatment results in the conversion of phenolic groups to *o*- and *p*-quinone structures. Ring cleavage can give rise to muconic acid type structures.

At present, no single procedure exists for the determination of all the types of ethylenic structures present in lignins. Two methods have been used with some success. One is based on the catalytic hydrogenation of the double bonds in cinnamaldehyde and cinnamyl alcohol structures and measurement of the UV difference spectrum. The other uses the difference in UV absorption in neutral and alkaline solution to measure the amount of phenolic stilbene structures. In both procedures, prior reduction of carbonyl functions using lithium aluminium hydride has to be carried out. Recently, a method for the determination of quinonoid ring systems has been reported (Argyropoulos 1995). It is based on an addition reaction with trimethyl phosphite and subsequent ^{31}P NMR measurement.

Carbonyl Groups. An early method for the determination of the carbonyl content of lignin preparations is based on the use of hydroxyl amine (Chen 1992d). The carbonyl group reacts with hydroxyl amine hydrochloride, forming an oxime and releasing one mole of hydrochloric acid. The total carbonyl content of a lignin preparation can be determined by titration of the hydrochloric acid liberated in the reaction. Alternatively, the carbonyl content may be determined by reduction with sodium or potassium borohydride. The borohydrides, which are soluble in weak alkali without decomposition, effectively reduce aldehydes and ketones. After the reaction, excess sodium borohydride is decomposed by addition of dilute sulfuric acid, and the volume of hydrogen thus liberated is measured volumetrically. The carbonyl content is calculated from the amount of consumed borohydride.

The contents of conjugated carbonyl groups have been estimated by measuring the difference in UV absorption resulting from the reduction of a conjugated carbonyl group to the corresponding alcohol. The use of quantitative

UV is problematic in lignin chemistry. The exact structure and the molar absorptivity of the chromophores in lignin are not known, and the model compounds used as standards are arbitrarily selected. For the determination of conjugated aldehyde groups, an NMR method has been developed that uses the integrals of the aldehyde proton signals that disappear on reduction with borohydride (Sect. 4.6.2).

Carboxylic and Sulfonic Acid Groups. Only small amounts of carboxyl groups are present in protolignin, but such groups are generated during alkaline pulping and bleaching. Both the residual lignin in kraft pulps and the soluble lignin in kraft black liquors contain carboxyl groups. No methods are available for the direct determination of the carboxyl groups separately in the lignin part because of the presence of other carboxyl-bearing constituents, mainly polysaccharides in pulp, and hydroxy acids in black liquor (Chap. 1). Spectroscopic methods have been used for the determination of carboxylic acid groups in isolated lignin samples (Sect. 4.6.2; Dence 1992c). The residual lignin in sulfite pulps contains sulfonic acid groups that can be determined selectively in the presence of carboxyl groups (Chaps. 1 and 6). As for the soluble lignosulfonate samples isolated from sulfite spent liquors, perhaps the most reliable methods for their determination are those based on conductometric titration (Beatson 1992).

4.8 Lignin Model Compounds

Lignin model compounds are "simple" molecules that are synthesized for the purpose of studying the reactivity and spectral properties of specific structural units in lignins (Fig. 4.3). The role of lignin model compounds as reference compounds is crucial in almost all types of analytical lignin work. The advantage of using model compounds is that the simplified compounds with a defined structure can mimic given structural units in the lignin, without the interference from the other structures present in a real lignin sample. The spectroscopic and reactivity data obtained from a number of such model compounds can then, with the necessary amount of caution, be combined and applied to the analysis of a "real" lignin sample. All important degradation methods (Sect. 4.6) have been optimized and cali-brated using model compounds, and the use of spectroscopic methods also depends heavily on data accumulated from model studies, for example UV data (Aulin-Erdtman and Sandén 1968 and preceding work) and the NMR database developed at the USDA Forest Products Laboratory in Madison (Ralph et al. 1994). The choice of model compounds for a given purpose is usually made based on the problem at hand; in some cases quite simple models are sufficient, at other times more ambitious synthetic efforts are needed (Zakis 1980).

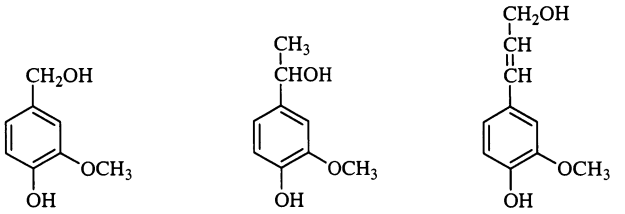


Fig. 4.29. Monomeric lignin model compounds

Monomers. For analysis of structural features associated only with the aromatic ring, and reactions of the phenolic hydroxyl groups, monomeric model compounds such as those shown in Fig. 4.29 are often used. The usefulness of the data from studies using such very simple models is in most cases quite limited.

Dimers and Trimers. Most researchers use model compounds that contain two or three aromatic nuclei. This makes it possible to mimic the chemical and spectroscopic properties of the inter-unit linkages in lignins (Fig. 4.3). The most important are, of course the dimeric model β-aryl ethers. Our knowledge, for instance, of the important reactions occurring during delignification in alkaline pulping processes is mainly based on work with model compounds. Figure 4.30 demonstrates the types of compounds that have been used as reference for the study of lignin analysis and reactivity.

Oligomers and Polymers. For biodegradation studies and other studies that focus on the behavior of polymeric lignin, reference compounds with high molecular weight are used. The best known example is the dehydrogenation polymer (DHP) that was introduced by Freudenberg to demonstrate the biosynthesis of lignin. The lignin precursor coniferyl alcohol is oxidized to produce a polymeric material that in many ways resembles lignin. The structure of DHP is not well known, however. A better understanding of the polymeric aspects of lignin reactivity is obtained by examination of synthetic oligomers (tetra-, penta-, and hexamers) (Kilpeläinen et al. 1994c) or lignin models prepared by attaching dimeric model compounds to an inert polymer backbone, such as polystyrene, polyvinyl alcohol, or polyethylene glycol (Shingo et al. 1995).

4.9 Determination of Macromolecular Properties of Lignin

Because the macromolecular properties are studied in solution, one experimental difficulty in the case of native lignin is its low solubility in most solvents. However, the solubility of lignosulfonates and kraft lignin is good in

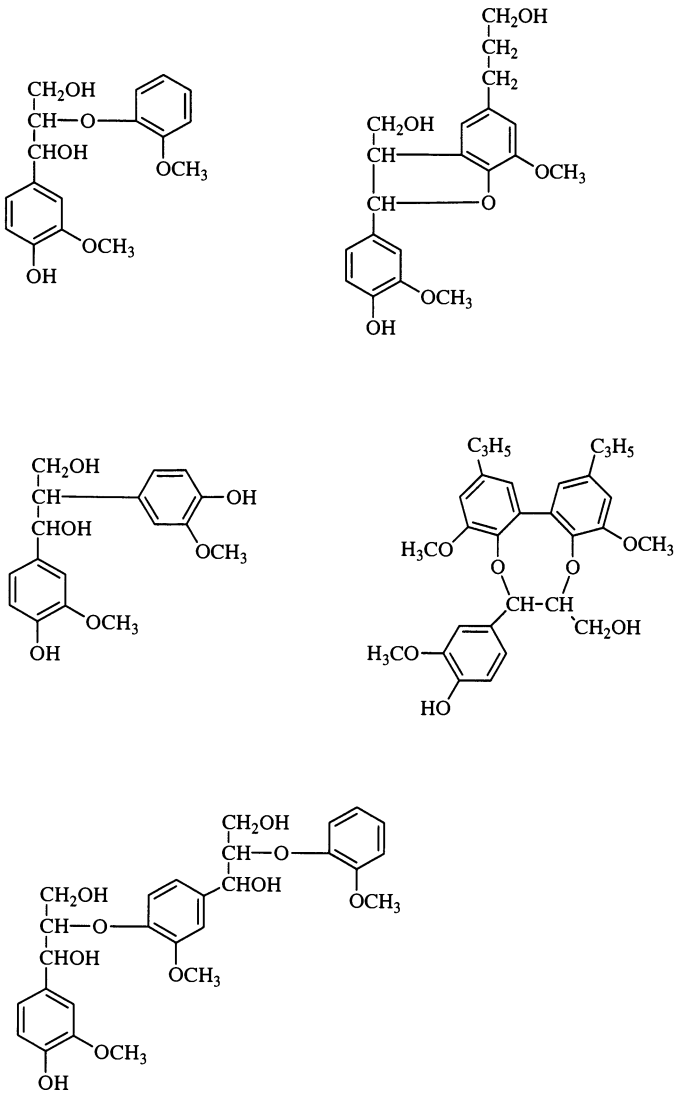


Fig. 4.30. Dimeric and trimeric lignin model compounds

water and sufficient in many organic solvents. In connection with their recovery from pulping liquors and utilization as by-products, much work has been carried out to study their polymer properties.

In addition to the molecular weight and molecular weight distribution, the other macromolecular properties of lignin are of interest. For example, based on the measurements of intrinsic viscosity, sedimentation, and diffusion rates, it has been shown that lignosulfonates and kraft lignin in solution

exist as a compact, spherical structure, quite different from that of cellulose (e.g., Sjöström 1993). Even if these types of polymer properties of lignin are important in connection with certain technical applications, they are not further discussed here because the lignin by-products are not included in this book.

The number-average molecular weight ($\overline{M}_n$) and weight-average molecular weight ($\overline{M}_w$) of lignin are determined by vapor pressure osmometry (Pla 1992a) and light scattering (Pla 1992b), respectively. An additional method, even if seldom applied to lignin, is ultracentrifugation (Brown 1967). The discussion is not extended to these tedious techniques, needing complex and expensive instrumentation. A comprehensive treatment can be found in the literature cited above.

Gel permeation chromatography (GPC), sometimes named size exclusion chromatography (SEC), is a modern and more convenient method. In an improved version of this system, the separation is made at a high pressure using small columns with high resolution efficiency. This type of technique is named high performance size exclusion chromatography (HPSEC). Additional separation methods include ultrafiltration (UF; Lin 1992a,b) and ion exclusion (IE; Alén et al. 1991), but these techniques are mainly used for fractionation of lignosulfonates and kraft lignin.

GPC on Sephadex has been applied since the early 1960s by Forss and his coworkers to the fractionation of both lignosulfonates and kraft lignins in aqueous media (for references, see Gellerstedt 1992b). The original Sephadex gels were successively improved and modified, resulting in new products suitable for fractionation in organic solvents. Today, organic gels based on cross-linked polystyrene resins having a uniform particle size, are available. In addition, inorganic materials, mainly based on porous silica particles, are used. With the polystyrene gels, organic solvents should be used, which is a limitation in the case of lignosulfonates and kraft lignin, which are water-soluble.

The most common organic solvent used in HPSEC is tetrahydrofuran (THF). The solubility of most lignin samples in THF is, however, so limited that it must be increased by derivatization. In the case of isolated wood lignin samples, such as MWL, a sufficient solubility is obtained after acetylation or methylation of the hydroxyl groups. Residual pulp lignin samples, especially those isolated from different (oxidative) bleaching stages, additionally contain carboxylic acid groups. These samples can be treated with diazomethane, which converts the carboxylic acid groups to methyl esters with a simultaneous etherification of the phenolic hydroxyl groups. Silylation has also been used for derivatization. An example of the HPSEC of lignin samples is shown in Fig. 4.31.

The most reliable, albeit laborious, way for calibrating the column system in HPSEC is to measure the molecular weight of the individual fractions by independent methods, including osmometry and light scattering. Another and a much more convenient alternative is to calibrate the system by means of commercial polystyrene or protein samples representing different ranges of

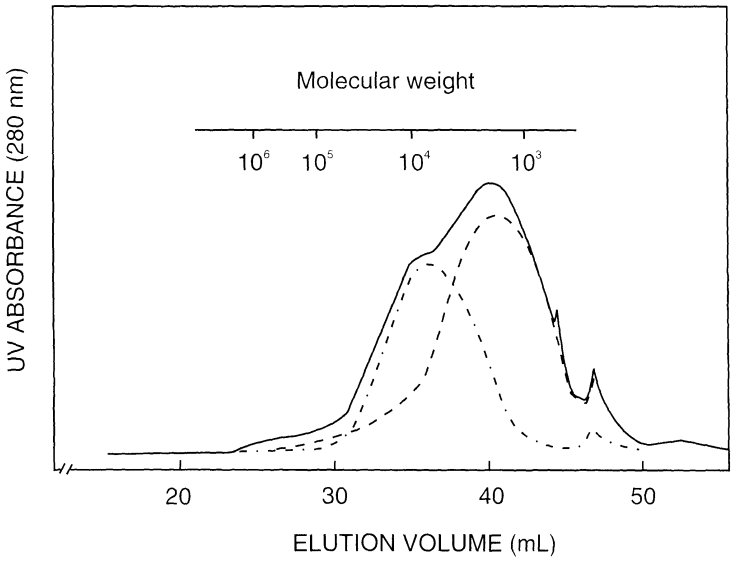


Fig. 4.31. High-performance size-exclusion chromatography of silylated samples of pine kraft lignin (——), birch kraft lignin (- - - -), and milled wood lignin from spruce (·····). Adapted from Gellerstedt 1992b

known molecular weights. However, because lignin samples behave differently in comparison with these standards in HPSEC, the molecular weight values obtained are only more or less rough estimates.

The choice of a suitable combination of gel and eluent in GPC and HPSEC is critical. For example, in GPC of lignosulfonates, the shape of the chromatogram is dependent on the ionic strength of the eluent and the amount of sample introduced to the column. Adsorption and partition effects also interact in GPC of non-ionic samples. Figure 4.12 shows an example of GPC conditions where the side effects have been largely eliminated.

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5 Extractives

B. Holmbom

5.1 Introduction

The term extractives is mostly used to denote the hydrophobic (lipophilic) components both in wood and in pulp and paper samples that are extractable with neutral solvents (Hillis 1962; Ekman 1979; Saranpää and Nyberg 1987; Fengel and Wegener 1989; Rowe 1989; Kai 1991; Sjöström 1993). These water-insoluble, lipophilic extractives are also called wood resin. However, although some of the phenolic extractives, mainly occurring in heartwood, can be dissolved in organic solvents, they are not usually considered true wood resin components. Sometimes the term extractives is used more broadly, to also include water-soluble substances, thus covering essentially all wood components other than cellulose, hemicelluloses, and lignin.

Wood extractives can be classified according to their morphological site and function in the tree (Table 5.1). This scheme also classifies the extractives according to their polarity and solubility in different solvents.

The lipophilic, water-insoluble extractives (wood resin), include the substances soluble in neutral solvents, such as dichloromethane, diethyl ether, and hydrocarbon solvents such as hexane. Wood resin is composed mainly of resin acids and other diterpenoids present in the resin canals of softwoods and of fats and steryl esters present in parenchyma cells that occur in both softwoods and hardwoods. Structures of some representative wood resin components are shown in Fig. 5.1.

There are considerable differences between extractives in softwoods and hardwoods, and also between wood species. Resin acids occur only in softwoods, and the proportions between individual resin acids differ between wood species. The fatty acid composition also differs between softwoods and hardwoods, and to some extent also between wood species. Furthermore, the climate influences the fatty acid composition within the same species. Trees growing in a cold climate produce a higher proportion of dienoic and trienoic fatty acids, whereas trees in a warm climate have a higher proportion of saturated and monoenoic fatty acids. Sugars and starch exhibit a pronounced seasonal variation, with high levels during the winter. However, lipophilic extractives do not have significant seasonal variations.

Large differences are found in extractives in the radial direction in wood stems. Pines have many more resin acids in the heartwood than in the sapwood. Heartwood also contains fewer triglycerides and more free fatty acids than the sapwood. Sugars, starch, phenyl glycosides, and certain phenols

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Table 5.1. Classification of wood extractives

Location in the wood	Resin canals (oleoresin)	Parenchyma cells	Heartwood	Cambium and growth zone	Ascending water Sap
Major compound classes	Resin acids, monoterpenoids, other terpenoids	Fats, fatty acids, steryl esters, sterols	Phenolic substances	Glycosides, sugars, starch, proteins	Inorganics
Main function in the tree	Protection	Physiological, food reserve, cell membrane components	Protection	Biosynthesis, food reserve	Photosynthesis, biosynthesis
Solubility					
Alkanes	+++	+++	0	0	0
Diethyl ether, dichloromethane	+++	+++	++	0	0
Acetone	+++	+++	+++	++	+
Ethanol	++	++	+++	+	+
Water	0	0	+	+++	++
Occurrence	Softwoods	All wood species	Mainly softwoods	All wood species	All wood species

+++: easily soluble, ++: soluble, +: slightly soluble, 0: insoluble.

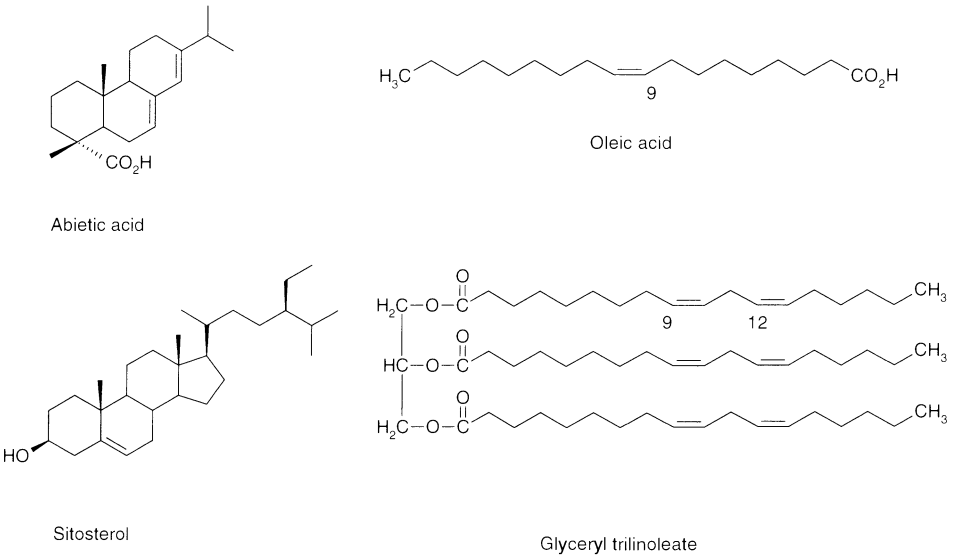

Fig. 5.1. Structures of major wood resin components

are concentrated in the outer part of the sapwood. Heartwood contains specific heartwood components, mostly of phenolic character. Vertical differences in the stem can also occur. For instance, pine trees have the highest resin acid content in their heartwood just above and under the ground.

The wood resin components are of special interest in pulping and papermaking, because they can give various negative effects, by process disturbances and by lowering the product quality (Table 5.2).

This chapter focuses on analytical methods for the lipophilic extractives in wood, pulp, and paper (Browning 1967, 1977; Assarsson 1969; Zinkel 1975; 1989; Easty and Thompson 1991; Sitholé 1992; Wallis and Wearne 1996). Analysis of pulp and paper mill deposits (Sjöström and Holmbom 1988; Sitholé et al. 1996; Sundberg et al. 1997) are also briefly discussed. Analysis of extractives in spent liquors from pulping and bleaching is treated in Chapters 7 and 8, respectively.

5.2 Sampling and Sample Pretreatment

Generally, the sampling strategy is determined by the objectives of the analysis and by the material to be analyzed. Sampling of wood is especially critical for analysis of extractives, because of the large differences in extractives between various wood tissues. Pulp and paper samples are generally rather

Table 5.2. Effects of lipophilic resin components in pulping and papermaking

Effect	Major responsible component groups
Process disturbances	
Foaming	Resin and fatty acid soaps
Deposits in kraft mills	Ca soaps of fatty acids, steryl esters, hydrocarbons
Deposits from mechanical pulps	All resin groups, but especially triglycerides and fatty acids
Wet-end chemistry disturbance	Colloidal pitch droplets, mainly composed of triglycerides, fatty acids
Effluent toxicity	Resin acids, diterpene aldehydes and alcohols, sterols
Product quality impairment	
Lower sheet strength	Triglycerides, fatty acids
Lower water absorbance	All hydrophobic components
Lower friction	Fatty acids, triglycerides
Taste and odor	Unsaturated fatty acids (after oxidation)
Allergic reactions	Oxidized resin acid products

homogeneous. However, fines, which are rich in parenchyma cells, usually contain more extractives than long fiber fractions.

Samples of fresh wood should be frozen immediately after sampling to avoid enzyme-induced changes and oxidation of the extractives. Wood samples can be stored at −20 to −24°C for weeks or even months. Wet pulp and paper samples are prone to microbial deterioration and should also be stored in a freezer, if stored for longer periods than a few days. Dry pulp and paper samples do not very easily deteriorate, although oxidation of sensitive resin and fatty acids can occur. For determination of volatile extractives (monoterpenoids) special precautions are needed to avoid evaporation losses during both sampling and sample storage.

Wet samples usually need to be dried before extraction. Samples must be dry especially when water-immiscible solvents, such as alkanes, are used for extraction. Drying before grinding facilitates the grinding. Grinding is normally not necessary for pulp samples.

For wood samples, the following treatment is recommended before extraction: (1) preparation of splinters (like wooden matches) from the wet wood, (2) freeze-drying of the splinters, and (3) grinding, e.g., to a particle size less than 0.4 mm (40 mesh screen). Grinding should be performed with a minimum of heat generation. Small amounts of clean dry ice can be added to prevent excessive heating.

Samples from an alkaline process contain the salts of fatty and resin acids. In order to achieve complete extraction of these acids from solid samples, they must be treated with acid before extraction. For instance, in the new SCAN standard CM-49:93 it is recommended that the pulp sample is treated with acetic acid solution (pH 2.5) for 10 min at room temperature. After dewatering and drying at 40°C overnight, the sample is ready for extraction.

5.3 Extraction

In most instances, it is necessary to isolate the extractives from the samples before their analysis. Extraction of solid samples is usually made with Soxhlet or Soxtec extractors (Sitholé et al. 1991). The choice of solvent is critical. Extraction of liquors, process waters, and effluents is usually made by solvent-water extraction, but various sorbents can also be used (see Chap. 9). Water samples can be extracted like solid samples after water removal, e.g., by freeze-drying. This procedure is recommended especially when the liquors contain much fibers or particles which have a tendency to adsorb resin components. The fibers and particles may also be separated and analyzed separately. In the case of deposits or resin products, such as tall oil, they are dissolved directly in the solvent.

5.3.1 Solvents

A large number of solvents have been used for extraction of wood, pulp, and paper samples. Each solvent or solvent mixture produces its own specific extract, not directly comparable with extracts obtained using other solvents. Therefore, standardization is important and it would be highly desirable to limit the number of solvents. Today, they still include the following (ratios are expressed on the volume basis): ethanol, ethanol:benzene (1:2), ethanol:toluene (1:2), acetone, acetone:water (9:1), dichloromethane as well as various alkanes.

A new SCAN standard method (SCAN-CM49:93) was issued in 1993, replacing dichloromethane with acetone for extraction of pulps. Dichloromethane is a good solvent, but has certain health and environmental hazards. Acetone has been widely used or extraction since the 1970s, and has also become the standard solvent in Canada (CPPA Standard G13 and G20).

Acetone, or even better acetone:water (9:1), is an effective solvent for common wood resin components (even for calcium salts of fatty and resin acids, as described below). It is inert and stable and is not harmful from a health and environmental point of view. It can be conveniently evaporated, leaving no water residue (azeotrope with water: 88.5% acetone and 11.5% water). Acetone of high purity is available at a moderate price.

Compared with dichloromethane, acetone has the disadvantage that it also extracts some hydrophilic components, such as simple sugars and phenyl glycosides. Consequently, the gravimetrically determined extract will not be a correct measure of the amount of nonpolar resin, i.e., lipophilic extractives. However, such a measure can be obtained by diluting the acetone extract solution with water, followed by extraction with a nonpolar solvent, such as hexane. The gravimetric value of the hexane extract corresponds well to the amount

of lipophilic, true resin. It is also possible to determine the lipophilic components (resin) in acetone extracts specifically in the presence of hydrophilic components by chromatographic analysis.

Fatty acids and resin acids form insoluble Ca soaps at a pH above 6–7 in the presence of Ca ions, and such Ca soaps are also commonly used as collectors for ink particles in flotation deinking processes. Recently, we have found in our laboratory (Sundberg et al. 1997) that such Ca soaps can be directly extracted by acetone in the presence of an excess of solid KH_2PO_4, which converts the Ca soaps to free acids, thus making the use of strong acid unnecessary.

Solvents other than acetone and acetone-hexane can be recommended only in special cases. Tetrahydrofuran (THF) is a better solvent than acetone for most synthetic polymers present in paper products. Many of such polymers are causing sticky deposits in deinking processes and on paper machines using recycled paper pulp. In cases where both sticky polymers and wood resin components occur, THF may be the solvent of choice. Of course, acetone cannot be used for extraction of liquors and process waters. In such cases, methyl *tert*-butyl ether (MTBE) can be recommended as solvent.

5.3.2 Extraction Techniques

Soxhlet extractors are available in different sizes, ranging from sample amounts from 0.5 g to several hundred grams. Standard methods recommend extraction of 10 g of wood or pulp in Soxhlet extractors using about 250 ml of solvent. The larger the Soxhlet extractor, the more time is needed for a certain number of extraction cycles. It will take about 4 h with 10 g of wood to obtain 16 extractions, which is recommended in standard methods with acetone as a solvent, whereas the same number of cycles is obtained in only 1 h with a small Soxhlet extractor using 15 ml of solvent for extraction of about 1 g of wood or pulp. The reason for extraction of as much as 10 g is that the extract yield is determined gravimetrically, requiring an extract amount of about 10 mg or more. However, if the gravimetric determination is substituted for more sensitive methods, e.g., GC determination, a small Soxhlet can be used to save both time and solvent.

Another way to reduce the amount of solvent and to make the extraction faster is to use a Soxtec extractor, where the sample is extracted by immersion in the solvent (Fig. 5.2). Instead of about 180 ml used in a standard Soxhlet extraction, only 50–100 ml is needed in a Soxtec extractor. The Soxtec system also includes automatic rinsing with clean solvent and evaporation of the solvent. The extraction time is only about 15 min plus about 1 h for rinsing, instead of at least 4 h when using standard Soxhlet extractors. The new SCAN method (SCAN-CM49:93) mentions Soxhlet and Soxtec extraction as equal alternatives. The Soxtec values have been found to be slightly lower than the

corresponding Soxhlet values for extraction of pulps, but when performing double extractions, practically identical values are obtained.

Soxtec extraction, but not Soxhlet, can be employed for extraction of Ca soaps of fatty acids with the use of potassium phosphate as mentioned above. Extraction of polymeric stickies in recycled pulp with THF is also more effective in Soxtec than in Soxhlet extractors. An alternative to Soxtec is simply to reflux the sample in a flask equipped with a condenser.

Supercritical fluid extraction (SFE) has been proposed as an alternative to a conventional solvent extraction for wood and pulp extractives (Björklund-Jansson et al. 1993). Because of a better solute diffusion and an effective mass transfer, the extraction is considerably faster with supercritical fluids than with ordinary solvents. However, the extraction equipment is much more expensive and the method is not well suited for routine use. Also, quite different results can be obtained from time to time when using the same medium (supercritical carbon dioxide which is the most common) depending on the pressure and temperature conditions during extraction. At optimal conditions SFE compares well with Soxhlet extraction.

Recently, a new extraction system called accelerated solvent extraction (ASE) has been developed. ASE combines elevated temperatures and pressures with the standard solvents used for Soxhlet extraction. ASE is much faster and

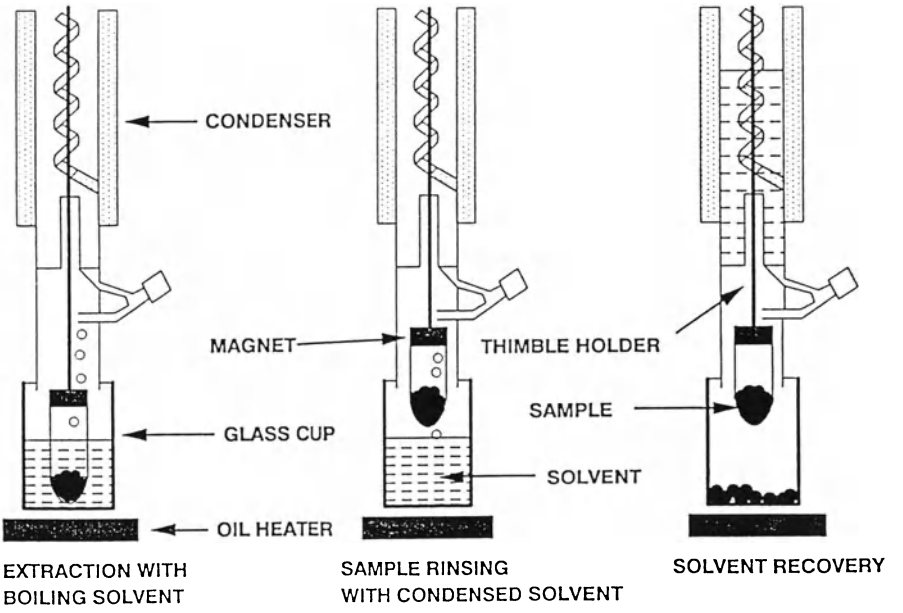

Fig. 5.2. Scheme of Soxtec extraction

requires considerably less solvent than traditional techniques. It is probably also useful for extraction of wood and pulp samples.

5.3.3 Solvent Evaporation and Drying of Extracts

Solvent evaporation under vacuum in rotaevaporators is a tedious operation. For small amounts of solvent, evaporation under a stream of inert gas, such as nitrogen, is more convenient, enabling evaporation of a larger number of samples in parallel.

To ensure that the extracts will be free of residual water, it is recommended that the extracts are kept for 10–15 min at 40°C in a vacuum oven. Residual water can also be removed by freeze-drying. Loss of volatile components, such as monoterpenes and partly also sesquiterpenes, cannot be avoided. However, other common wood resin components are not lost. Extracts should not be dried at higher temperatures since many extractive components are sensitive to oxidation and isomerization. However, use of an inert gas is not necessary during drying of extracts in a vacuum oven as described above.

5.4 Analysis of Extracts

Analysis of extractives can be made at three levels:

1. Gravimetric or other determination of total extractives,
2. Determination of different component groups, and
3. Analysis of individual components (sometimes preceded by a group separation).

Only a gravimetric determination of extractives may be sufficient for routine process and quality control in pulp and paper mills. However, with acetone replacing dichloromethane as the standard solvent, the amount of extractives is even less informative than before, since acetone is not very specific for lipophilic wood resin. Further determination of the hexane-soluble part of the acetone extractives gives a more appropriate value for the wood resin content.

Quantitative determination of the main resin groups, i.e., triglycerides, steryl esters, sterols, fatty acids, and resin acids, provides adequate and relevant chemical information, and is sufficient in most process studies. However, for research purposes as well as for more detailed chemical process studies, information of individual components is often needed. The composition of individual fatty acids, resin acids, sterols, etc., is particularly important in studies related to the environmental or health effects of the extractives.

5.4.1 Determination of Component Groups

Component groups in extracts can be determined by several chromatographic techniques: gas chromatography (GC), high-performance liquid chromatography (HPLC), size-exclusion chromatography (SEC), supercritical fluid chromatography (SFC), and thin-layer chromatography (TLC). Quantitative analysis of extracts is possible also by ^{13}C NMR. In addition, Fourier transform infrared spectroscopy (FTIR) has been used, although the quantitation is not very easy.

Gas Chromatography. The extremely high resolution achieved by capillary open-tubular columns, makes GC an excellent technique for complex multicomponent mixtures such as extractives. GC also has an ideal detector, the flame ionization detector (FID), which is sensitive and reliable, has a wide linear range, and gives nearly the same response for different organic compounds. The combination of GC and mass spectrometry (GC/MS) is an excellent technique for component identification in complex mixtures.

The GC and GC/MS techniques were developed in the 1960s and 1970s and they are now mature, reliable methods. The invention of fused-silica capillary columns around the year 1980 initiated a development that made easy-to-handle and reliable columns commercially available from several producers. The development of powerful, inexpensive computers and easy-to-use data handling programs has solved the problem of chromatogram peak area measurement and the handling of data from a large number of samples of multicomponent mixtures.

The need for derivatization has been considered a disadvantage of GC compared to LC techniques. However, it is not a very time-consuming step in the analysis of extractives compared with the time needed for sample drying, grinding, extraction, and solvent evaporations.

GC is particularly powerful for analysis of individual resin components (Holmbom 1977; Foster and Zinkel 1982; Ekman and Holmbom 1989; Han and Zinkel 1990; Lee and Peart 1991; Sitholé et al. 1992; Dethlefs and Stan 1996; Wallis and Wearne 1997). In addition, GC can also provide convenient quantitative component group determination, including steryl esters and triglycerides, by employing short columns. This was demonstrated in the 1970s, using short packed columns (Chapman et al. 1975). Later, short thin-film capillary columns were adopted. Recently, we have in our laboratory (Örså and Holmbom 1994) developed this technique further by simplifying derivatization and by improving quantitative accuracy and precision. The entire analytical procedure, including small-scale Soxhlet extraction, is outlined in Table 5.3.

The resolution obtained with such a short column is of course not the best, but the main groups of lipophilic extractives are separated well enough to enable their quantitative determination (Fig. 5.3). Until now, the method has

Table 5.3. A convenient procedure for the determination of resin component groups in wood, pulp, and paper samples. GC procedure as described by Örså and Holmbom (1994)

1. Extraction	Preferably with acetone at a small scale (0.5–1 g of ground and dried wood, pulp or paper) employing mini-Soxhlet extractors. Four standards added to the solvent before extraction.
2. Evaporation	About 1 ml of the solvent is taken out into a test tube and evaporated. Solvent evaporation in a stream of nitrogen. The extract is transferred with a small amount of solvent to a vial used in GC autoinjectors, and is evaporated again.
3. Silylation	Addition of 80 μl BSTFA and 40 μl TMCS. Held in an oven at 70 °C for 20 min.
4. Injection	Autoinjection, on-column (no split), temperature-programmed injector: 80–340 °C.
5. GC analysis	5 m/0.53 mm i.d. column coated with a 0.15 μm film of dimethyl polysiloxane 100–340 °C, FID at 340 °C. Entire analysis cycle 35 min.
6. Calculation of results	Peak area integration and calculation of results (in, e.g., mg/g dry sample weight) with a computer and appropriate chromatographic data handling software. Manual check of the integration baseline.

been used mainly for the analysis of extractives in spruce and pine woods and mechanical pulps, as well as process waters and effluents from paper mills. However, it is also applicable to other wood species and other types of pulps, as well as to the analysis of pitch deposits. Exchange of internal standard compounds may be needed when investigating other wood species. For instance, with birch containing betulinol, this compound cannot be used as the internal standard.

High-Performance Liquid Chromatography. HPLC has been used for group analysis of extractives in both size-exclusion (SE) and reversed-phase (RP) modes (Suckling et al. 1990). A fairly good separation of wood resin groups can be obtained in the SE mode using cross-linked polystyrene resin columns and tetrahydrofuran (THF) as the solvent (Fig. 5.4).

One problem is the overlapping of sterols with fatty acids. The quantitative determination of the extract composition is not as straightforward as in GC. The refractive index detector usually employed is not as universal as the FID and the detector response is much more dependent on the compound structures. This means that the group composition will notably affect the response, and correction factors can not be unambiguously determined. Also, the use of

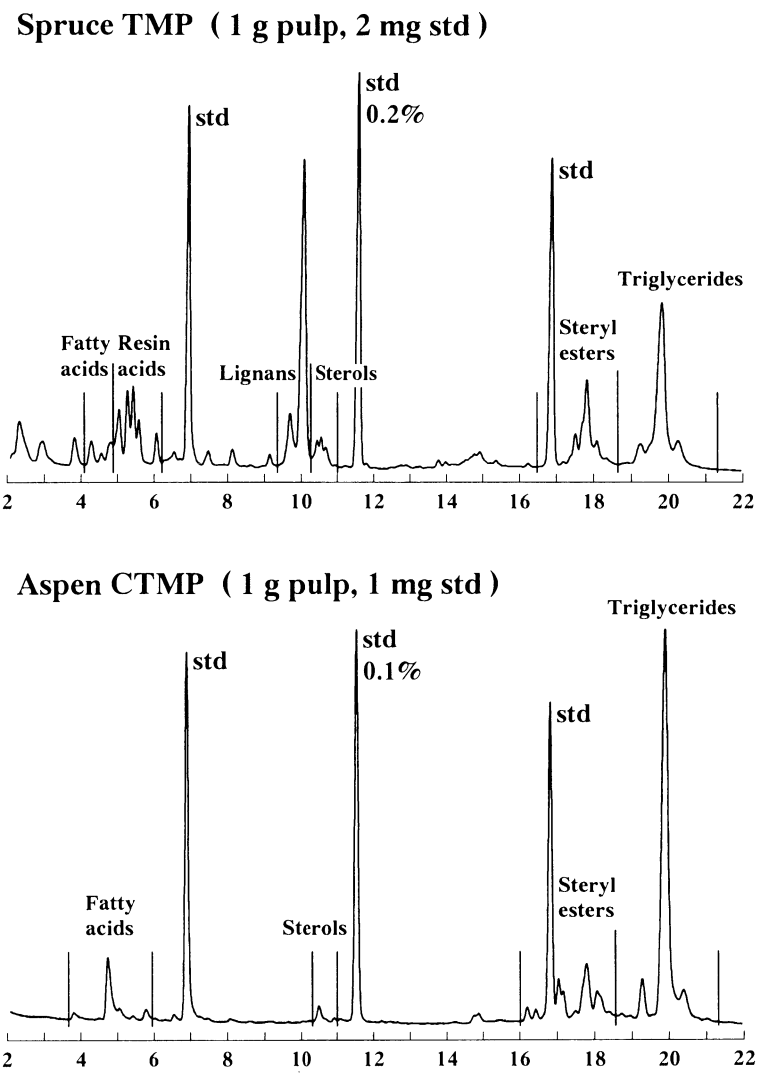

Fig. 5.3. GC on a short capillary column of acetone extracts of spruce TMP and aspen CTMP. Extraction, derivatization, and GC conditions as described in Table 5.3

quantitative internal standards is difficult. Thus, the HPLC-SEC technique must still be considered as an only semiquantitative method.

HPLC-SEC can be made without derivatization, although methylation will improve the separation of fatty and resin acids. HPLC-SEC enables the isolation of separated fractions for further analysis of individual components.

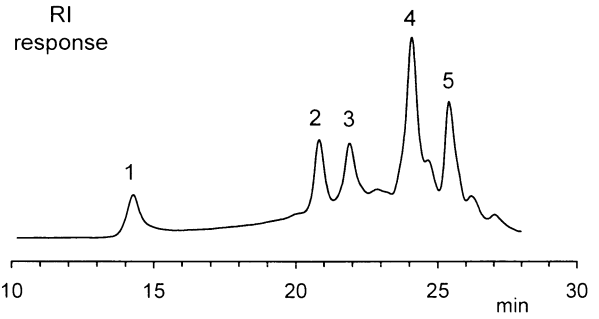

Fig. 5.4. Analysis of a THF extract of a deinked pulp (mainly from household waste paper) with HPLC-SEC. Columns: G 3000 HXL, G 2500 HXL, and G 1000 HXL, in series. Eluent: THF, 1 ml/min. Detector: refractive index. Peak identities: *1* synthetic polymers, *2* triglycerides, *3* steryl esters, *4* fatty acids and sterols, and *5* resin acids

Possible polymeric constituents can also be detected and separated for further investigation, e.g., by pyrolysis-GC (Py-GC) or IR. This is of particular interest in studies related to recycled paper and deinking. In such cases, THF is recommended as the extraction solvent. Pitch deposits in paper mills also often contain polymeric material, especially if deinked pulp is used. A scheme employing HPLC-SEC for preparative group separation enables detailed characterization of small samples (0.5–1 mg) of pitch deposits, including both polymeric material and wood resin components (Fig. 5.5).

RP-HPLC has also been applied to the analysis of wood resin and pitch deposits. The amounts of dehydroabietic acid and other resin acids, linoleic acid, oleic acid, and triglycerides can be determined in a single analysis using gradient HPLC on a C18 column (Fig. 5.6). One problem here is the overlapping of steryl esters and triglycerides.

There are, however, problems with this technique with respect to quantification. There are no good detectors available. The mass detector used is quite insensitive and it has a very limited linear range with a wide variation in response to the different resin components. There are also significant daily variations of the response, making it necessary to redetermine the response curves every day. The use of quantitative internal standard compounds is also not as straightforward as in GC. It seems that the RP-HPLC technique at the present level of development cannot compete with GC for the quantitative determination of wood resin. However, it may be an alternative or complement to HPLC-SEC and TLC for preparative separation of certain extractive groups for further analysis by other techniques.

Supercritical Fluid Chromatography. SFC is a technique having similarities to both GC and HPLC (Björklund-Jansson et al. 1993). As in GC, FID can be used. SFC also enables rapid direct analysis of wood and pulp extracts without hydrolysis. Derivatization is not needed. The separation is principally similar

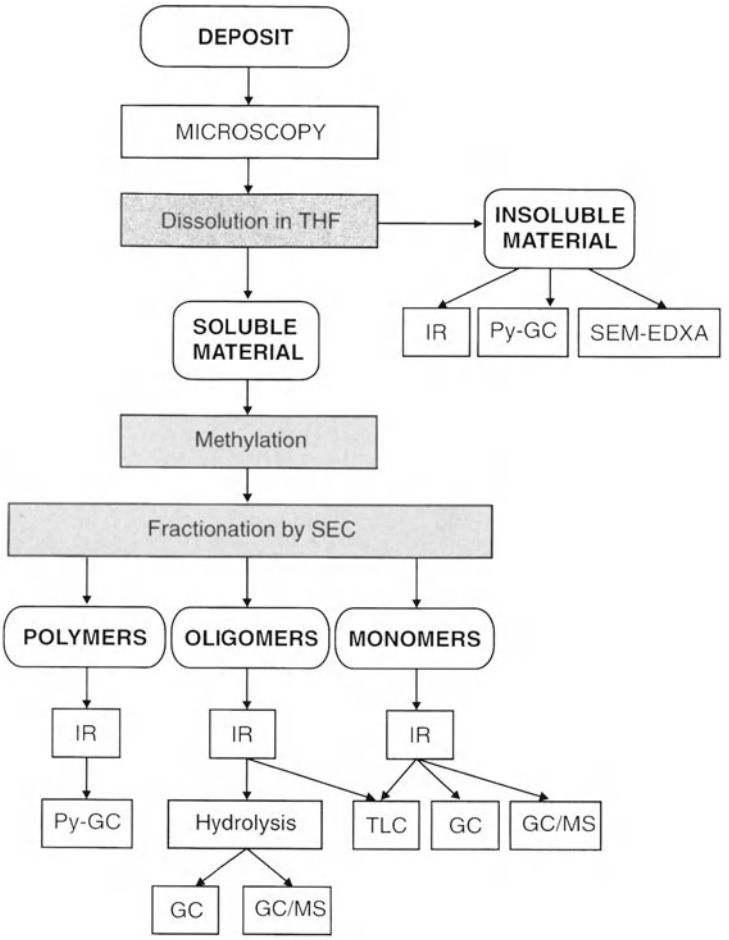

Fig. 5.5. Scheme for fractionation and characterization of pitch deposits in pulp and paper mills. (Sjöström and Holmbom 1988)

to that of GC. However, steryl esters and triglycerides are not separated (Fig. 5.7).

Thin-Layer Chromatography. TLC is an inexpensive and convenient technique providing a clear visual image of the sample composition (Laamanen 1984; Sandström et al. 1996). It is also fast because many samples can be analyzed in parallel. TLC is also well suited for preparative isolation of component groups prior to GC to HPLC.

TLC on silica plates gives excellent separation of groups in wood resin, namely hydrocarbons, steryl esters, diterpene aldehydes, triglycerides, fatty and resin acids, and various terpene alcohol and sterol components. Detailed

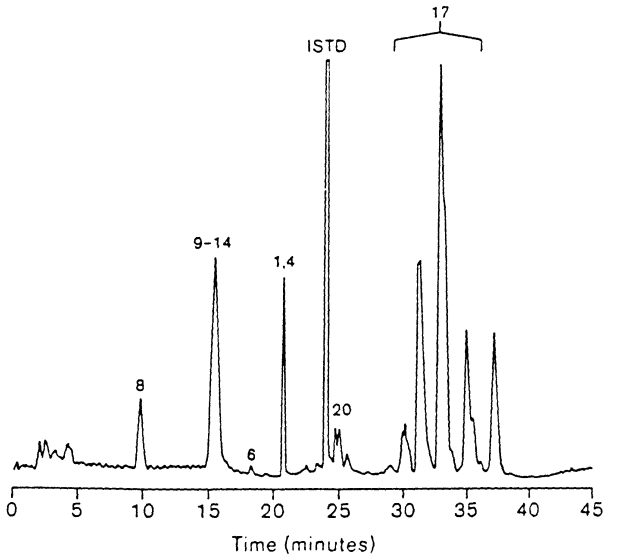

Fig. 5.6. RP-HPLC chromatogram of *Pinus radiata* sapwood resin. Peak identities: *8* dehydroabietic acid, *9–14* other resin acids, *6* linoleic acid, *1,4* palmitic and oleic acid, *20* sitosterol, and *17* triglycerides. (Suckling et al. 1990)

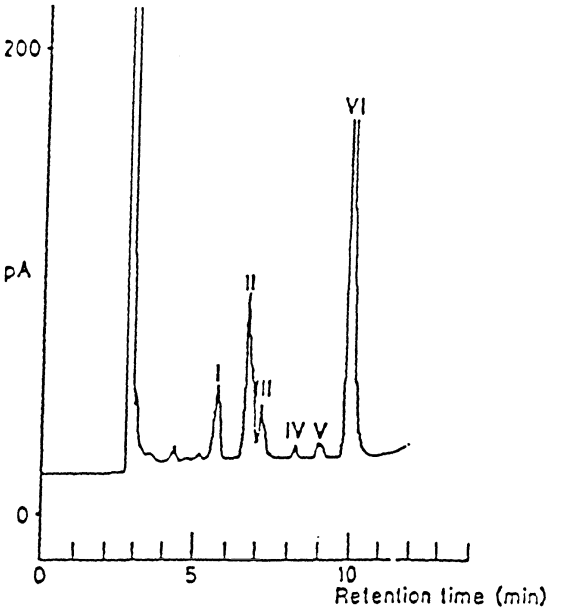

Fig. 5.7. Supercritical fluid chromatogram (SFC) of a hexane extract from a newsprint mill white water. Peak identities: *I* fatty acids, *II* resin acids, *III* oxidized resin acids, *IV* sterols, *V* diglycerides, and *VI* triglycerides and steryl esters. (Björklund-Jansson et al. 1993)

analysis of individual resin components can be obtained by preparative isolation of the component groups followed by analysis of individual components by GC or HPLC. Accurate quantitative analysis can be performed if appropriate internal standards for each group are added to the sample. This technique has been successfully applied to obtain very detailed characterization of wood extractives (Fig. 5.8).

It has recently been demonstrated (Sandström et al. 1996) that fatty and resin acids can be well separated on silica TLC plates by using the solvent system dichloromethane:methanol:ammonia (80:19:1).

Quantitative determinations can be made by TLC using special detection and densitometric techniques. However, only a few efforts have been made to develop the quantitative TLC of extractives, and it has not been used generally for quantitative analysis of extractive groups.

Solid-Phase Extraction. A similar fractionation to TLC using SPE was recently presented (Chen et al. 1994). Wood or pulp extracts are sorbed on extraction cartridges filled with aminopropyl-substituted silica material and different groups of extractives are eluted by a sequence of solvents. The extracts can be divided into fractions containing waxes + steryl esters (including hydrocarbons), triglycerides, fatty acids, fatty alcohols + sterols + diglycerides, and monoglycerides. After evaporation of the solvent, the separated fractions are quantified by weighing.

The procedure includes the use of three columns and several solvent mixtures and a large number of elution and evaporation steps, and it is thus not very simple and fast. At the end, five fractions are obtained, containing mainly

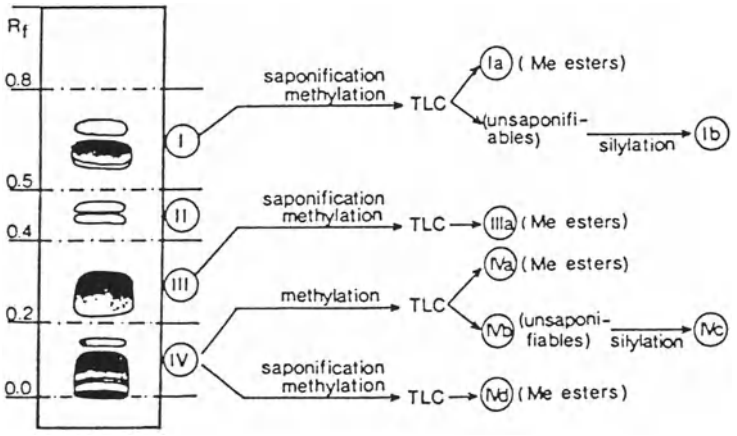

Fig. 5.8. Fractionation of extractives by TLC on silica gel plates. Solvent system: petroleum ether :diethyl ether (85:15 by vol.). Fraction identities: *I* hydrocarbons and oxides, *Ia* fatty acids from esterified sterols, *Ib* sterols, *II* diterpene aldehydes and resin acid methyl esters, *IIIa* triglyceride fatty acids, *IVa* free fatty and resin acids, *IVb* free diterpene alcohols, *IVc* free sterols, and *IVd* mono- and diglyceride fatty acids. (Ekman 1979)

one class of compounds. However, the fatty and resin acids are not separated. The quantitative reproducibility is also questionable since it is based on weighing of fractions, most of which are smaller than 10 mg. The concluding statement (Chen et al. 1994) that the SPE procedure is fast, cheap, versatile, and accurate and does not require highly trained personnel, seems to be an overstatement.

NMR Spectroscopy. The group composition of resin samples can be determined also by ^{13}C NMR (Suckling and Ede 1990). This nondestructive technique can give the amounts of fatty acids, resin acids, triglycerides, and fatty acid esters in wood and pulp extracts. Vanillin was used as an internal standard for quantification. Determination was based on the carbonyl signals in the region (170 to 200 ppm) from resin acids, fatty acids, and fatty acid esters, and the glycerol signals from triglycerides (55 to 75 ppm).

However, NMR analysis requires a large amount of extract and the analysis time per sample is long. With 1 g of extract the analysis can be performed in 1 h, but if only 100 mg is available the analysis time is typically between 16 and 24 h. The instrument must be operated in a quantitative mode, meaning that for extractives there is about a 15-s delay between the pulses. It is also costly to use an extremely expensive modern FT-NMR instrument for many hours. Furthermore, only a skilled operator can perform the NMR analyses.

5.4.2 Analysis of Individual Components by GC

The superb separation on capillary GC columns makes GC the preferred method for determination of individual resin components. During the era of packed GC columns, from the end of 1950s to the beginning of the 1970s, many methods for fractionation of extractives were developed, such as (1) successive extractions with a series of solvents of increasing polarity, (2) separation of acidic components from neutral ones by partition between organic solvent and alkaline water, and (3) use of ion exchange chromatography for separation of weak acids, strong acids, and neutral components. However, such fractionations are tedious and time-consuming and also involve risks of chemical alteration of sensitive components, as well as loss of material, causing errors in quantitative results.

Now, with the high-resolution capillary columns, such prefractionations are in most cases obsolete. All important fatty and resin acids, various diterpenoids, fatty alcohols, and sterols can be analyzed simultaneously in one analysis on standard GC columns. Prefractionation by other chromatographic techniques, such as TLC or HPLC-SEC, are needed only in special cases, for instance, if the fatty acid compositions of steryl esters and triglycerides are of interest.

Derivatization Methods. Although sterols and fatty alcohols, and even fatty and resin acids, have been successfully analyzed directly by GC as such, derivatization is recommended to ensure a reliable quantification.

Formation of trimethylsilyl ethers is the most common derivatization technique for alcohols. A combination of the reagents *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) and heating for 20 min at 70 °C effects a quantitative silylation of sterols and terpene alcohols, including betulinol, which has a sterically hindered and thus not very reactive hydroxyl group. Silyl ethers of such alcohols are stable in solution for days and even weeks.

Fatty acids can be easily methylated, even with acidic methanol or with BF_3 in methanol. In contrast, the carboxyl group of resin acids is sterically hindered and methylation requires stronger methylation agents. Diazomethane methylates resin acids well when dissolved in diethyl ether containing some methanol. However, nowadays most laboratories avoid diazomethane because of the severe health hazards involved. An alternative is to use trimethylanilinium/tetramethylammonium hydroxide (TMAH) and to perform in situ methylation in the injector. However, the quantitative reliability of this derivatization has not been definitely proven.

Both fatty and resin acids are smoothly converted into trimethylsilyl esters with the same silylation agents as the alcohols, e.g., BSTFA and TMCS. Silyl esters are more sensitive to hydrolysis than the silyl ethers, and several researchers have discouraged the use of silyl ethers for fatty and resin acids. However, we have in our laboratory used silylation in parallel with diazomethane methylation in derivatization for GC on nonpolar dimethyl polysiloxane columns. The retention times are not the same, but the common fatty and resin acids present in wood and pulp extracts can be separated equally well as silyl esters (Fig. 5.9). Levopimaric acid, which is overlapped as methyl ester, is resolved as silyl ester. The quantitative results have also been proved to be identical. However, silylated esters have a limited stability and it is recommended that silylation is made within 24 h before the GC analysis. The use of silylation rather than methylation also allows the determination of resin acid methyl esters which are present in low amounts in softwood resin.

Pentafluorobenzyl (PFB) esters of fatty and resin acids have been suggested for environmental studies of water and sediment samples. With these esters, an electron-capture detector (ECD) instead of FID can improve the detection sensitivity and selectivity significantly. PFB esters are also suitable for GC/MS analysis.

GC Columns. Nonpolar dimethyl polysiloxane capillary columns of 15–30 m length provide appropriate separation of all main fatty and resin acids, fatty and terpene alcohols, and sterols. Some researchers have used other polysiloxane column types, such as DB-5 and other vinyl-substituted polysiloxane types. There are, however, no apparent advantages of such slightly more polar

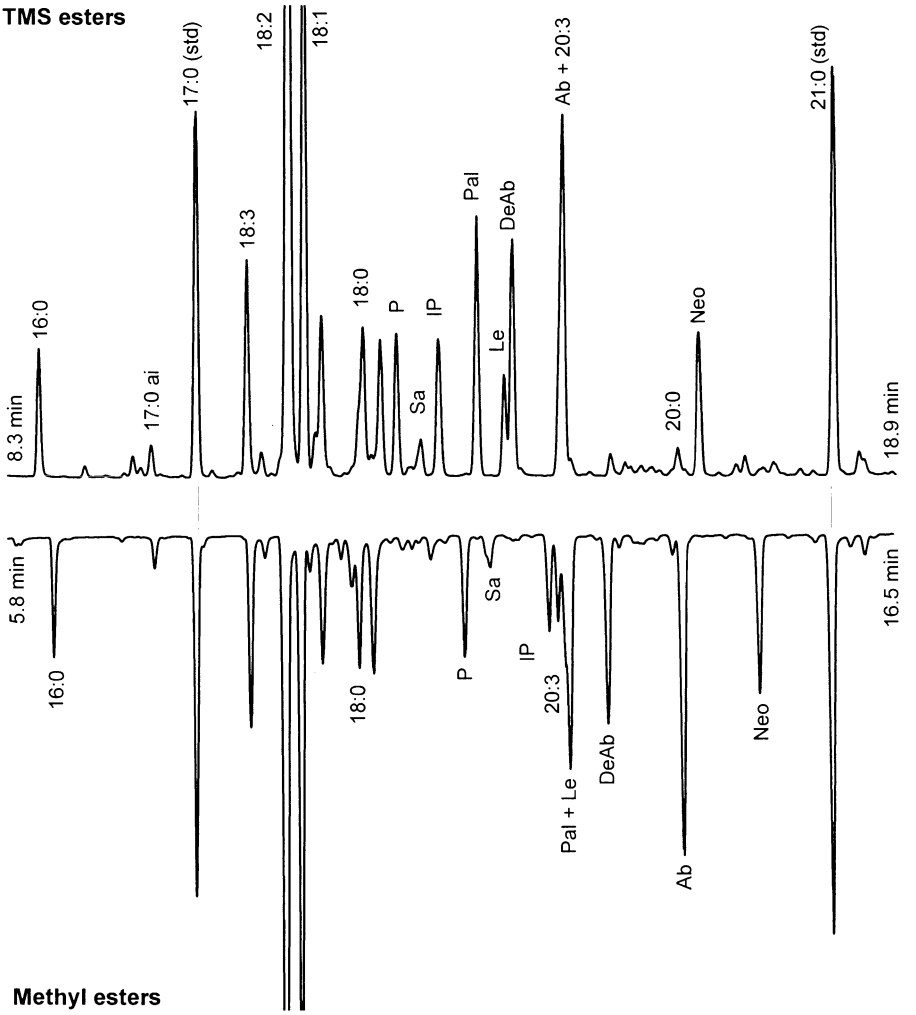

Fig. 5.9. GC analysis of the fatty and resin acids for a Finnish kraft (sulfate) soap as trimethylsilyl and methyl esters. GC column: 25 m/0.32 mm i.d. HP-1 (dimethyl polysiloxane). Temperature program as in Fig. 5.10, *upper chromatogram*. Peak identities: Fatty acids: *16:0–20:0* saturated acids, *17:0ai* anteiso-branched 17:0 acid, *18:3* pinolenic, *18:2* linoleic, *18:1* oleic, *20:3* 5,11,14-eicosatrienoic acids. Resin acids: *P* pimaric, *Sa* sandaracopimaric, *IP* isopimaric, *Pal* palustric, *Le* levopimaric, *DeAb* dehydroabietic, *Ab* abietic, *Neo* neoabietic acids

columns, on the contrary, they only make the comparison between different studies difficult. Only in special cases are other columns needed. For instance, separation of all conjugated fatty acids formed in the kraft pulping process and the resin acid isomers formed in tall oil distillation, requires the use of polar columns, such as butanediol succinate (BDS). For separation of *trans*-isomers

of fatty acids, extremely polar polysiloxane phases substituted by cyanopropyl groups are recommended. For analysis of silyl esters, only nonpolar silicone phases should be employed.

Effects of Column Oven Temperature. If components of interest overlap, it is worth trying another column oven temperature. A change in temperature will change the column separation slightly. In some cases it can even reverse the elution order of components. Although this effect is stronger with polar columns, it is notable also with nonpolar polysiloxane columns. The effect is significant in analysis of fatty and resin acid mixtures (Fig. 5.10). Completely overlapping fatty and resin acid pairs can be well separated by small changes in the column temperature. Analysis of a sample at different temperatures offers the possibility of distinguishing between fatty acids and resin acids, since the resin acids will move forward in the chromatogram relative to the fatty acids with increased temperature. This temperature dependence can accordingly make the peak assignment either more difficult or more simple. Change in oven column conditions, and even change in the carrier gas flow, will give changes in the chromatogram peak pattern.

Aspects on Quantification in GC. Although integration of peak areas in chromatograms is nowadays fairly straightforward and reliable, thanks to effective computers and advanced chromatography software, all problems of quantification in GC have not been solved.

Severe quantitative discriminations can occur in the injection. Because low-volatile components can be left in the syringe, the so-called solvent flush technique is recommended. Automatic injection is also to be preferred over manual injection with a syringe. Injection with splitting of the sample between the column and an outlet (so-called split injector) commonly gives severe discrimination of low-volatile components, such as of sterols relative to fatty and resin acids. Non-split on-column injection is much more reliable. Use of several internal standard components with different volatilities (e.g., both a fatty acid and a sterol-type compound) can partly compensate for the errors. Another problem is that different components have different detector responses. However, with FID there are reasonably small differences between individual fatty and resin acids. Here again, use of several different internal standard components can compensate for some of the differences.

Severe quantitative errors can occur if compounds are irreversibly adsorbed onto active sites in the column, which is indicated by tailing of the peaks. Tailing peaks demand corrective measures, such as cleaning of the injector or replacing the column.

Gas Chromatography/Mass Spectrometry. Identification of components separated by GC is most conveniently made by GC/MS. Identification of mass spectra are easily made by comparison with spectra in data bases, which are available both in electronic form or in printed form. These data bases also

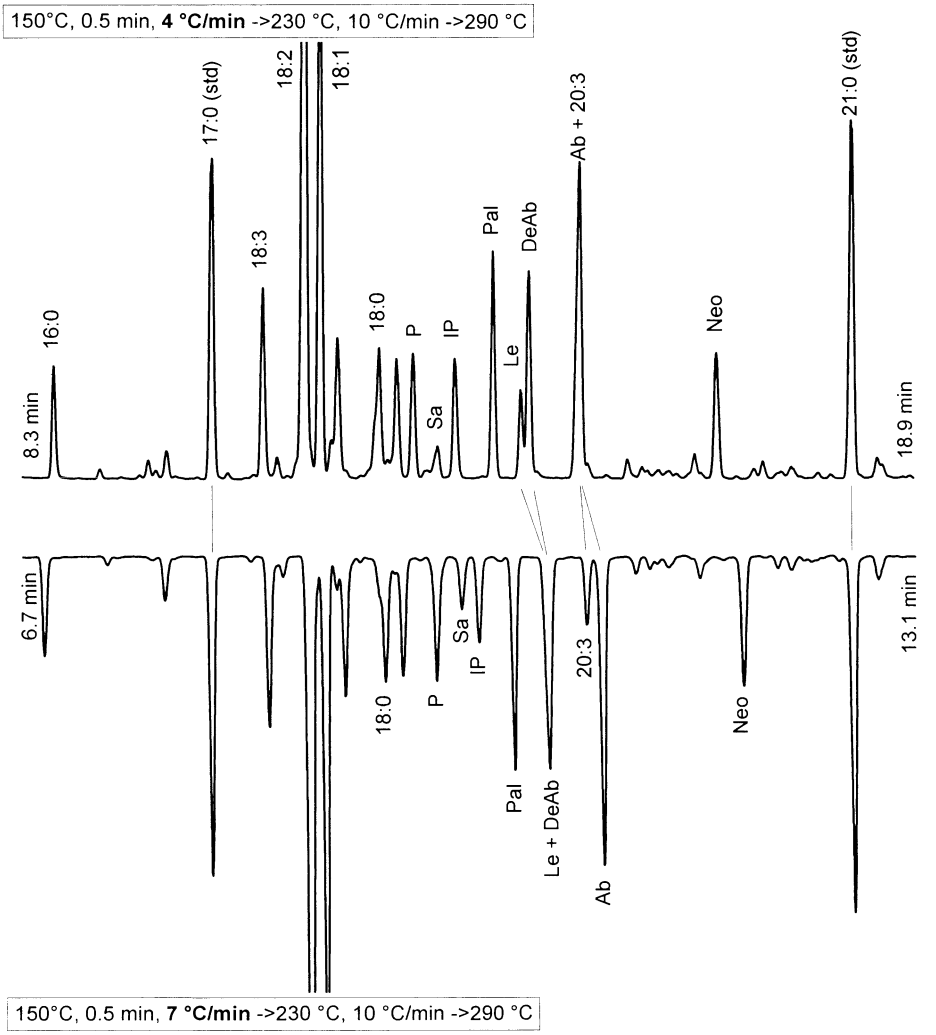

Fig. 5.10. GC analysis of fatty and resin acid trimethylsilyl esters with different temperature programs. GC column and peak notations as in Fig. 5.9

contain spectra of most common extractive components, although some components, such as some resin acids are still missing.

GC/MS is not possible with the short wide-bore GC columns used for group separation of extractives as described above. Here, identification needs to be performed by other means, such as comparison of retention times with those of appropriate reference substances.

5.5 In-Situ Analysis of Extractives and Spots in Pulp and Paper Samples

Extractives remain as impurities in various pulp and paper products in small amounts, commonly less than 0.1%. Extractives are often found as spots and specks in the products and also as deposits on papermaking machinery parts, such as wires, felts, suction boxes, cylinders, etc. In order to avoid process disturbances caused by extractives, rapid chemical characterization with direct in-situ methods which do not require complicated and time-consuming extractions, are important.

IR instruments are common in industrial laboratories, and IR is often used for characterization of pulp and paper mill deposits. IR spectra provide structural information about the constituents, but quantitative analysis of various constituents is usually not possible. However, the interpretation of spectra of complex mixtures, such as deposits, is usually not easy. FTIR microscopy is an interesting nondestructive technique for a direct characterization of small spots in pulp and paper products (Sweeney 1989). Spots as small as 10 μm can be examined.

Py-GC is a rapid direct technique which is excellent for the analysis of small spots containing impurities or added chemical agents in paper products (Hardell 1993; Ishida et al. 1994). Because the extractives contain carboxylic acids, a technique called simultaneous pyrolysis methylation (SPM) has proved valuable. Methylation is carried out directly on the pyrolysis filament by the addition of tetramethylammonium hydroxide. It is possible to distinguish between softwood and hardwood resins, and various sizing agents, including ordinary rosin, fortified rosin, and alkyl ketene dimers (AKD).

The papermaking properties of fibers are largely associated with the physical structure and chemical composition of the surfaces of the fibers forming the paper sheet. For example, accumulation of extractive components on the fiber and paper surfaces affects the sorption properties and the friction. The distribution of wood resin in paper sheets can be visualized by staining the resin with a fluorescent dye followed by examination with confocal laser scanning microscopy (Willför et al. 1997). Surface-specific determination of extractives on fiber surfaces is possible with X-ray photoelectron spectroscopy (XPS), also called ESCA (electron spectroscopy for chemical analysis), and secondary ion mass spectrometry (SIMS). These techniques are treated in Chapter 6.

5.6 Summary and Concluding Remarks

Nonpolar, lipophilic wood extractives (wood resin) can be analyzed after extraction with suitable solvents. Recently acetone has been adopted as a stan-

dard solvent for extraction of wood, pulp, and paper samples. However, acetone also extracts fairly polar compounds, and the acetone extract amount as such is not a correct measure of wood resin content.

GC using short columns is the method of choice for rapid, quantitative determination of the different groups of lipophilic extractives in wood, pulp, and paper and also in pulp and paper mill process waters. Other techniques can be recommended only in special cases, such as when preparative separation of compound classes are required for detailed group analysis, when high polymers (higher than triglycerides) need to be analyzed, and when simplicity and low costs are a must.

HPLC in the SEC mode is of particular interest in analysis of pitch deposits and resin in recycled pulp and paper which also contain polymeric material. Extracts can be visually and semiquantitatively compared by TLC, which is fast and without the need of complex, expensive instrumentation. TLC is also an excellent technique for preparative separation of compound groups for further detailed study by GC.

GC is the only technique giving complete separation of individual resin components. All important fatty and resin acids, various diterpenoids, fatty alcohols, and sterols can be analyzed as trimethylsilyl derivatives simultaneously in one GC analysis on standard nonpolar polysiloxane columns.

Extractives in spots and specks in pulp and paper products as well as in various deposits can be determined, at least qualitatively, directly without solvent extraction by FTIR and Py-GC. Extractives on fiber surfaces can be determined by ESCA or SIMS.

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6 Direct Characterization of Chemical Properties of Fibers

P. Stenius and T. Vuorinen

6.1 Introduction

This chapter describes analytical methods that are applicable for the characterization of the chemical composition of fibers as such, without a previous separation of the components or component groups from each other. Although the details of the structure and composition generally can be obtained only to a limited extent, these direct methods possess definite advantages. For example, a minimum of preparation is required. Often samples can be analyzed more or less directly and, in some cases, the lateral and vertical distribution of different chemical structures in the fibers can be determined. The methods can be roughly divided into the following groups:

1. "Dry" methods for the characterization of structural features of fiber constituents. These methods include different spectroscopies: nuclear magnetic resonance (NMR), infrared (IR), Raman and ultraviolet/visible (UV/Vis) as well as pyrolysis gas chromatography (Py-GC).
2. "Dry" methods for the surface analysis of fibers. By far the most important of these methods are electron spectroscopy for chemical analysis (ESCA), also known as X-ray photoelectron spectroscopy (XPS), and secondary ion mass spectroscopy (SIMS).
3. "Wet" methods for the determination of the amounts of different components and functional groups in the fibers as a whole or on the fiber surfaces by using both chemical and enzymatic treatments.

The discussion of each method starts with a description of the principle of the method, followed by some examples of its application to fiber analysis. The chapter ends with a table in which the different properties of fibers are listed and methods are suggested for the determination of each property.

6.2 Spectroscopic Determination of Bulk Composition

6.2.1 CP/MAS ^{13}C NMR Spectroscopy

In previous chapters, it was shown how the chemical structure of hemicelluloses, lignin, and extractives can be analyzed in solution by NMR spectroscopy.

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Firm signal assignments have become possible due to the signal resolution of modern high field NMR instruments and the development of multidimensional techniques. However, it is not always possible nor even desirable to dissolve the wood components prior to their analysis. In such cases, solid state NMR spectroscopy can be applied. Although the resolution of this technique is relatively low in comparison with solution state NMR spectroscopy, it may still give valuable information about the chemical and especially the physicochemical properties of wood.

The main reason for the broadening of NMR signals in the solid state is the angular dependence of the carbon-proton dipolar coupling. In a solid material a chemical bond may have different, fixed orientations relative to the external magnetic field of the NMR spectrometer (Fig. 6.1). The magnitude of the local field depends on the orientation of the bond which means that in the solid state a certain chemical structure generates a wide distribution of local fields. In solution, these variations are averaged due to fast molecular motions.

The angular dependence of the local field B_{loc} of a dipolar C–H bond is given by

$$B_{loc} = \pm\frac{h\gamma_H}{4\pi r_{C-H}^3}(3cos^2\theta_{C-H} - 1), \tag{6.1}$$

where h, γ_H, θ_{C-H}, and r_{C-H} denote the Planck constant, the gyromagnetic ratio of the proton, the angle between the external magnetic field and the dipolar

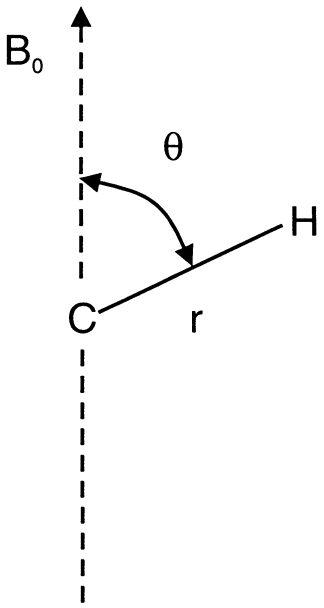

Fig. 6.1. Scheme of a dipolar C–H bond in an external magnetic field showing the parameters that affect the local magnetic field at the ^{13}C nucleus

bond, and the internuclear distance of the bond, respectively. From Eq. (6.1) it can be calculated that in the solid state the dipolar coupling may broaden the ^{13}C signals by ± 20 kHz, which typically covers the whole chemical shift range of solution state ^{13}C NMR spectroscopy. This means that acceptable solid state ^{13}C NMR spectra can usually be obtained only with a high-power proton decoupling.

Equation (6.1) shows that the local field is zero, if the angle between the dipolar bond and the external field is $\pm 54.7°$ or $\pm 125.3°$. If the solid sample is spun at a high rate, the dipolar bonds are artificially oriented (anti)parallel to the spinning axis. If, moreover, the spinning axis is at the "magic" 54.7° angle relative to the external magnetic field, the dipolar broadening can, in principle, be removed. However, the spinning rates needed are impracticably high.

On the other hand, magic angle spinning can be applied for the removal of chemical shift anisotropy caused by orientation of non-coupling polar structures in the solid state. The magnitude of the chemical shift anisotropy of carbon nuclei depends on their functionality, but is typically one or two orders lower than the dipolar broadening. The angular dependence is similar to that given by Eq. (6.1) but in this case, magic angle spinning (MAS) at a few kHz will remove the chemical shift anisotropy. Indeed, ^{13}C NMR spectra have almost exclusively been collected by using both proton decoupling and magic angle spinning together with cross-polarization that transfers part of the proton magnetization to carbon nuclei and thus increases the low sensitivity of the ^{13}C nuclei (CP-MAS technique).

Although the angular dependencies of dipolar coupling and ^{13}C chemical shifts are in many cases unwanted phenomena, they can be advantageously exploited in the analysis of spatially ordered materials such as crystalline compounds. Because the orientation of hemicelluloses, lignin, and especially cellulose is non-random in wood cell walls, ^{13}C NMR spectroscopy could, at least in principle, be applied for the analysis of the degree of ordering (Hatfield et al. 1987).

By applying both high-power proton decoupling and magic angle spinning it becomes possible to collect spectra of wood and pulp samples with a resolution of ≈ 1 ppm at 50 MHz. This resolution is generally too low for a detailed structural analysis of the chemical wood components. The solid state spectra may, however, reveal fine structures that are not observable in solution. For example, in the spectra of native celluloses, fine splitting is observed that indicates the presence of several allomorphs (Van der Hart and Atalla 1984; Newman 1994; Larsson et al. 1997). The unequivalent signals have been assigned to different crystalline or amorphous forms.

A major factor that is responsible for the variations in chemical shifts of the C-1, C-4, and C-6 signals of cellulose samples is rotation around the carbon-oxygen bond. Rotations around the interglycosyl bonds naturally result in different chain conformations and thus the C-1 and C-4 chemical shifts are indicative of chain conformation. The chemical shift of C-6, on the other hand,

may indicate specific hydrogen bonding with other hydroxyl groups in the same or neighboring chains.

By comparing the ^{13}C NMR spectra of crystalline glucose derivatives with X-ray diffraction data it has been possible to correlate the chemical shifts of the C-1, C-4, and C-6 atoms with the torsion angles of the corresponding carbon-oxygen bonds (Fig. 6.2) (Horii et al. 1983). These relationships are also very useful for the characterization of chain configurations of hemicelluloses. Thus, xylan, when sorbed on cellulose fibers, seems to adopt a chain configuration that is similar to that of the cellulose (Mitikka et al. 1995).

The strength of solid-state ^{13}C NMR spectroscopy is that it can give information about ordering of the chemical components of wood and pulp. We have already seen that such information can be extracted from the effects of dipolar C-H coupling and chemical shift anisotropy on carbon chemical shifts and from the relationships between torsion angles and chemical shifts of oxygen substituted carbon atoms. Still another information source is offered by the relaxation rates that correlate with the mobility of molecules.

For a successful relaxation analysis, it is necessary to assign certain signals that are typical of each chemical component of wood. For lignins, the aromatic carbon resonances (120–160 ppm) and resonances from methoxyl groups (56 ppm) can be applied in all cases. Some of the wood polysaccharides are

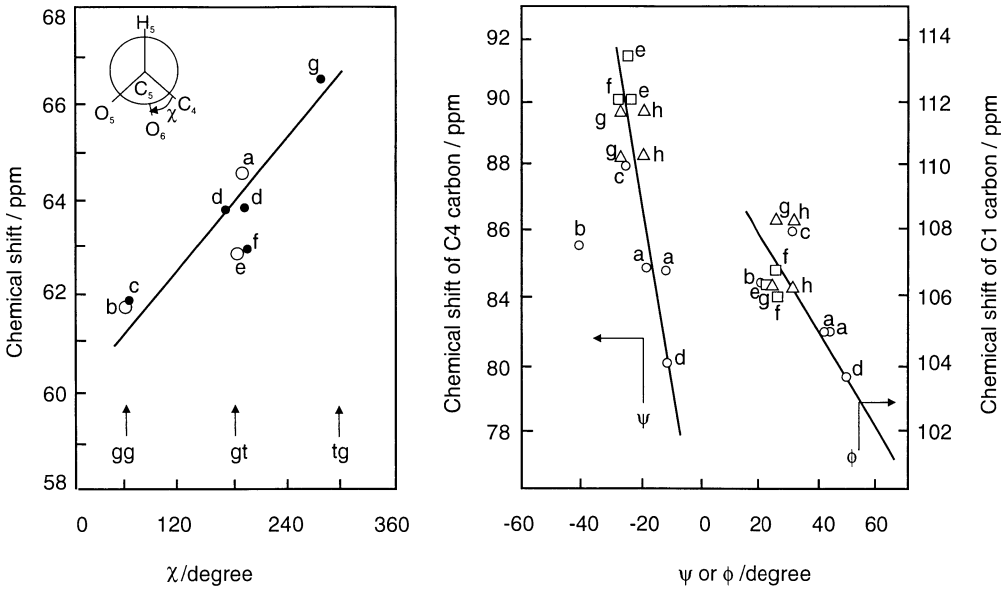

Fig. 6.2. Correlation of the ^{13}C chemical shifts of C1, C4, and C6 carbon atoms of α-D-glucopyranosyl (*filled symbols*) and β-D-glucopyranosyl residues (*open symbols*) in several saccharides with torsion angles around C1–O1 (Φ), C4–O1 (ψ), and C5–C6 (χ), respectively. (Horii et al. 1983)

acetylated, which means that the resonances from the acetyl group (21 and 173 ppm) are specific for these hemicelluloses. In most pulping or bleaching processes, the hemicelluloses are deacetylated. Therefore, alternative assignments are needed. For glucomannans, the assignment can be based on the anomeric mannose resonance at 102 ppm compared with the anomeric glucose and xylose resonances at 104–106 ppm. The C-4 atoms of cellulose resonate at specific chemical shifts of 89 (crystalline cellulose) and 84–85 ppm (amorphous cellulose). Similarly, the (ordered) xylan in wood and pulp shows the C-4 resonance at 83 ppm.

For the analysis of wood and pulp, the most important method applied has been rotating frame proton relaxation measurements (Newman and Hemmington 1990), although carbon relaxation can be utilized as well. In wood all (crystalline) cellulose relaxes slowly, whereas some of the hemicelluloses and lignin relax much faster (Fig. 6.3). During delignification, the situation changes so that most of the fast relaxing hemicelluloses are removed, while part of the crystalline cellulose begins to relax much faster. The origin of the changes in relaxation rates remains uncertain but the probable explanation is that in moist samples it is mainly due to direct interaction with water. In this case the relaxation phenomena can be correlated with the ultrastructure of the porous cell wall.

It has been speculated that the spin diffusion between the cell wall components would level off differences between the relaxation rates, but the fast relaxation of the residual lignin of pulps contradicts this theory.

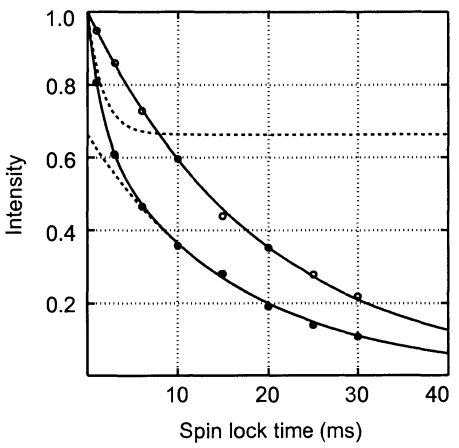

Fig. 6.3. Description of the mobility of acetylglucuronoxylan (*filled symbols*) in fresh birch wood by $T_{1\varrho}$ measurements of the acetyl group by CP/MAS ^{13}C NMR spectroscopy. Separation into slow and fast relaxing components (*dashed lines*) indicates that part of the wood xylan is almost as ordered as cellulose (*open symbols*), whereas the other part is very mobile and probably in direct contact with water

In addition to information on the physicochemical structure of wood components the solid state, ^{13}C NMR spectroscopy reveals their chemical structure and composition. The applications in this field are evident. For example, CP/MAS ^{13}C NMR spectroscopy has been used in the analysis of lignin contents of wood and pulp samples, photodegradation of mechanical pulps, etc. There are also possibilities of editing spectra so that they only give information about certain types of compounds or carbon atoms. Editing according to relaxation rates gives access to subspectra of more and less mobile components.

Spin-locking can be applied for selective suppression of protonated carbon atoms. The resulting spectrum includes only nonhydrogenated carbon atoms. The technique can be applied to, e.g., structural studies of residual lignins of pulps (Leary et al. 1988).

6.2.2 FTIR Spectroscopy

All chemical bonds undergo stretching and bending, resulting in absorption at characteristic frequencies in the infrared region of light. A prerequisite for IR absorption is that it be accompanied by a change in the electrical dipole moment of the stretching or bending structure. Functional groups that have large permanent dipole moments, such as carbonyl and hydroxyl groups, give strong IR absorption, whereas nonpolar bonds absorb IR radiation only weakly (Schrader 1995).

Modern IR spectrometers operate in the Fourier transform mode (FTIR), which means that incident radiation covers a wide IR region and that the interferogram is monitored instead of the intensity of monochromatic light. The time domain of the interferogram is converted using a Fourier transformation to the frequency domain, i.e., to the conventional absorption spectrum.

The techniques available for FTIR spectroscopy of solid samples include transmission, diffuse reflectance, internal reflectance, and photoacoustical measurements (Friese and Banerjee 1995). Because scattering of light occurs at discontinuities of refractive index, good transmission measurements require the use of a medium that is transparent for IR radiation. The common technique is to use potassium bromide pellets containing finely divided samples. The technique is mostly not practical for wood and pulp samples because extensive comminution of the sample is needed.

In *diffuse reflectance FTIR spectroscopy* (DRIFT) the solid sample is irradiated with a focused IR beam and the diffusely scattered radiation is collected with curved mirrors (Fig. 6.4). IR absorption results in a decrease in intensity of the detected scattered radiation. The advantages of DRIFT are that minimum sample pretreatment is needed and that the incident beam can be

focused very sharply (<10 μm) under a microscope. This means that, e.g., small deposits on paper can easily be analyzed.

In principle, the spatial resolution of FTIR microscopy is good enough for the analysis of individual cells (fibers) but a surface analysis is not possible because the analytical depth of DRIFT (40–140 μm) is much greater than the cell wall of wood fibers (<10 μm). The resolution of modern FTIR microscopes is also less than would be needed for analysis of topological distribution of cell wall components from transections of wood.

FTIR comes closer to a real surface analysis when using internal rather than diffuse reflectance. The internal reflectance is usually brought about by the *attenuated total reflectance* (ATR) technique, in which the incident IR beam passes through an IR transparent "crystal" (e.g., of germanium), on both sides of which is a sheet of the sample (Fig. 6.5). On its path through the crystal, the incident beam reflects several times at the borders of the crystal and the sample. At each reflection point an evanescent wave penetrates into the surface layer of the sample, which then may absorb radiation at the characteristic frequencies. The amplitude of the evanescent wave is given by

$$E = E_o exp\left(-\frac{2\pi n_1 z}{\lambda} \sqrt{sin^2\phi - \left(\frac{n_2}{n_1}\right)^2} \right), \tag{6.2}$$

where E, n_1, n_2, ϕ, z, and λ denote the amplitude of the evanescent wave, refractive index of the crystal, refractive index of the sample, incident angle of the IR beam, penetration depth in the sample, and wavelength of the IR radiation, respectively. According to Eq. (6.2), the penetration (analysis) depth depends not only on the incident angle and the refractive index of the crystal but also on the radiation wavelength, which means that the analysis depth is different for different vibrational modes in the sample (Fig. 6.6).

A very critical requirement for successful ATR measurements is a good contact between the crystal and the sample. In practice, ATR is well suited for the analysis of surface coated papers, whereas special techniques are needed in preparation of uncoated paper sheets for ATR measurements.

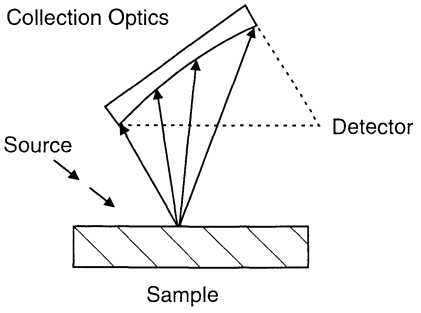

Fig. 6.4. Principle of diffuse reflectance FTIR spectroscopy (DRIFT). The solid sample is irradiated with a focused IR beam and the diffusively scattered radiation is collected with curved mirrors

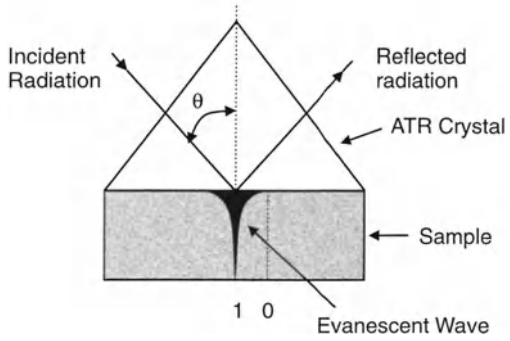

Fig. 6.5. Principle of attenuated total reflectance (ATR) spectroscopy. The incident IR (or UV-visible) radiation passing through the ATR crystal is internally reflected if $\sin\theta > n_2/n_1$. The evanescent wave will be attenuated at frequencies at which the sample absorbs radiation

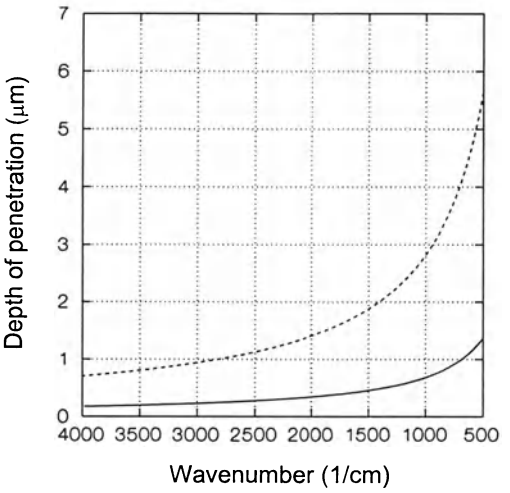

Fig. 6.6. Depth of penetration (E/E_0 = 0.37) in ATR spectroscopy of lignocellulosic material (n_2 = 1.6). Crystal material germanium (*solid line*, n_1 = 4.0) or KRS-5 (*dashed line*, n_1 = 2.4), incident angle 45°

A small ATR crystal can be assembled permanently in the focus of the FTIR microscope. The micro-ATR objective can be brought into contact with microscopic objects such as small deposits on paper or a single fiber. In practice, it is impossible to precisely control the contact area between the object and the crystal and therefore the analysis area will be affected by the size of the beam. In commercial micro-ATR accessories, the incident angle is built-in, which leaves less flexibility for the selection of the penetration depth.

In *photoacoustic spectroscopy* (PAS), the sample is irradiated with short pulses of IR light. Absorption of the chopped light induces oscillating thermal waves, which migrate to the surface of the sample. At the sample surface, the thermal waves cause pressure fluctuations or photoacoustic signals in the sur-

rounding gas. These signals are detected with a sensitive microphone. Most of the heat transferred to the gas phase is generated by thermal waves formed in a surface layer of thickness (L):

$$L = \sqrt{\frac{D}{\pi f}}, \tag{6.3}$$

where D is the thermal diffusivity of the sample and f is the modulation frequency of the light. The sampling depth can generally be varied in the region 1–100 μm through the selection of modulation frequency. In *constant velocity scan* spectrometers, the modulation frequency is wavenumber dependent, i.e., it is equal to the product of the wavenumber and the interferometer mirror velocity. In this case, L depends on the absorption frequency of the sample. On the other hand, spectrometers equipped with a *step scan* option can produce photoacoustic spectra at a constant penetration depth.

PAS is especially suitable for depth profiling of layered structures such as coated or printed papers. PAS has also been applied for surface analysis of single fibers and other microscopic objects. No sample pretreatment is needed, provided that the sample is dry. Because the sampling depth can be controlled, saturation due to strongly absorbing bands can be avoided – a problem that could be encountered in DRIFT.

IR absorption frequencies are characteristic of functional groups of chemical compounds. Aromatic skeletal vibrations at 1510 and 1600 cm^{-1} are commonly applied for quantification of lignin in wood and pulp samples (Faix 1992). Carbonyl stretching of carboxylate ions occurs at 1560–1590 cm^{-1}. Protonation of carboxylic acids shifts the carbonyl stretching band to 1620–1690 cm^{-1}, in which region conjugated carbonyl structures (ketones) of lignin also absorb radiation. Unconjugated ketones and esters have their carbonyl stretching bands at 1710–1740 cm^{-1}. Because of these overlaps, assignment of the carbonyl absorption bands needs to be confirmed with other techniques.

The backbones of wood polysaccharides are very similar and they cannot generally be quantified separately by FTIR spectroscopy, especially because their partially amorphous nature broadens the absorption bands (Tipson and Parker 1980). In deuterium oxide, hydroxyl groups of amorphous cell wall material is deuterated while those of crystalline polysaccharides, mainly cellulose, remain protonated. Because O–H and O–D stretching occur at different frequencies, deuteration enables assignment and quantification of the narrow O–H stretching bands of allomorphs of crystalline cellulose.

All wood components have a multitude of overlapping IR absorption bands, which makes quantification of single bands tedious and often unreliable. Frequently, the reliability of analysis is improved by application of statistical multivariate methods, including principal component analysis and partial least squares.

6.2.3 Raman Spectroscopy

While IR spectroscopy is based on absorption of radiation at those, and only those, frequencies that correspond to characteristic stretching and bonding frequencies of the sample molecules, Raman spectroscopy detects changes in the radiation frequency that are due to inelastic scattering of incident photons by the sample molecules (Fig. 6.7). In such scattering, the frequency of the photons is either decreased or increased by an amount that corresponds to a stretching or bending frequency of the molecular system (Schrader 1995).

The intensity of the scattered radiation is proportional to the fourth power of the incident radiation frequency. Hence, Raman sensitivity is enhanced by using short incident wavelengths. On the other hand, the frequency must be below the electronic absorption frequencies of the sample molecules. Typically, argon ion, krypton ion or helium-neon lasers are used as the radiation source. The advantage of using a laser source is that the radiation beam can be focused very sharply. The spatial resolution of Raman microscopes is $\approx 1\,\mu m$ which means that the resolution is good enough to analyze the chemical structure in different cell wall layers (M + P = 0.4–1.4 μm, S = 1–5 μm) in transections of wood (Atalla and Agarwal 1986).

Raman microscopes may operate confocally, thus enabling depth profiling of layered structures, such as coated or printed papers. Good resolution

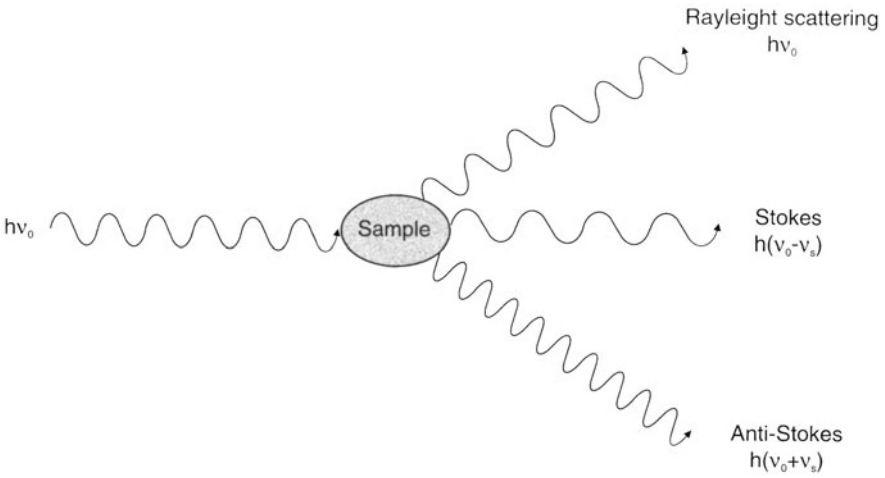

Fig. 6.7. Elastic and inelastic scattering of radiation by sample molecules. In elastic Rayleigh collisions, the frequency of the radiation remains unchanged (FTIR and UV-visible spectroscopy), whereas inelastic Raman scattering decreases (Stokes) or increases (anti-Stokes) the frequency of a photon. The change in the frequency corresponds to the energy difference between ground and excited states of the molecule (Stokes) or vice versa (anti-Stokes)

($\approx$1 μm) in the *z*-direction even makes depth profiling through single fibers possible.

Due to the nature of the Raman effect, all Raman instruments work in the diffuse reflectance mode. In comparison with FTIR, Raman spectroscopy is relatively insensitive because the probability of inelastic collisions is only $\approx 10^{-8}$. The Raman effect is strongest for relatively nonpolar bonds with symmetrical charge distributions, and therefore Raman and FTIR spectroscopies complement each other.

A great advantage of Raman spectroscopy is its insensitivity to water. Thus, in contrast to FTIR spectroscopy, wet pulp and wood samples can be analyzed. In FTIR spectroscopy the signals from the hydroxyl groups of wood polysaccharides dominate, while Raman spectroscopy is more sensitive to extractives, lignin, and the carbon-hydrogen bonds of polysaccharides (Fig. 6.8; Agarwal and Atalla 1995).

The laser beam may cause fluorescence, depending on the presence and structure of chromophores in the sample and the wavelength of the laser source. Unbleached chemical pulps are especially strongly fluorescent and difficult to analyze. FT Raman spectrometers that generally operate at lower frequencies than dispersive Raman instruments are regarded as being more suitable for such samples.

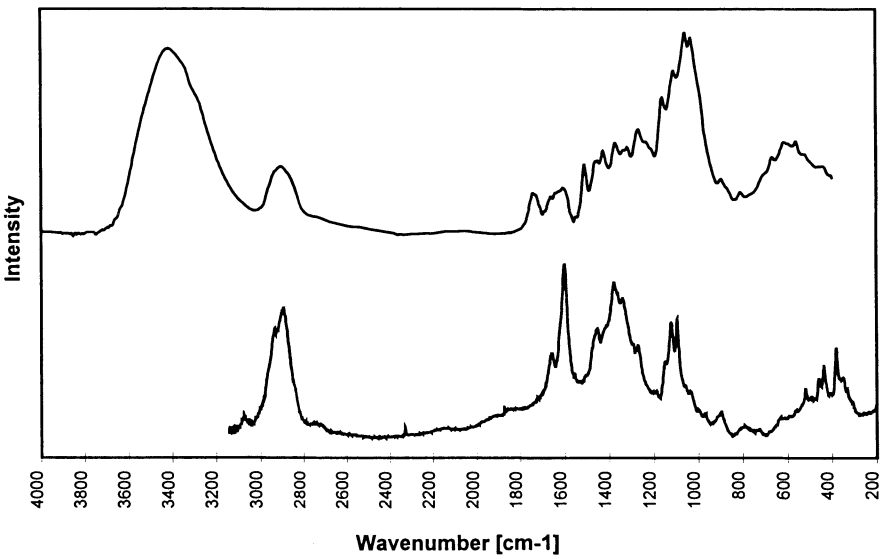

Fig. 6.8. FTIR (*above*) and Raman spectra (*below*) of a thermomechanical wood pulp. The FTIR spectrum shows strong bands of O–H stretching (3600–3100 cm^{-1}), C=O stretching (1750–1700 cm^{-1}), and C–O stretching (1150–1000 cm^{-1}), whereas the Raman spectrum includes strong bands of C–H stretching (3050–2800 cm^{-1}), C=C stretching (1650–1600 cm^{-1}), C–H deformation (1450–1300 cm^{-1}), and C–C stretching (1150–1050 cm^{-1}) Agarwal and Atalla (1995)

6.2.4 UV/Vis Spectroscopy

When entering an optically homogeneous particle, light is partly scattered from the particle and partly penetrates into it. The intensity of the penetrating light depends on the path length if the particle contains chemical structures that absorb at the wavelength of the light. If the absorption is incomplete, the particle transmits a portion of the light. Many solid materials, such as wood and paper, are optically inhomogeneous, which means that the material contains borders of alternating refractive indexes. In such a material, reflection and penetration or transmission occurs at each surface of discontinuity of the refractive index. If the internal surfaces are nonparallel, the light is diffusely scattered.

The color that a human senses for wood or paper depends on the wavelength distribution of the reflected or transmitted diffusely scattered light. The color of wood or paper may be an important criterion for its quality. Because the color is usually due to chemical chromophores, it is often of particular interest to analyze these chromophores by UV/Vis spectroscopy. The absorption of light in the UV and visible region originates from transitions in electronic state and therefore UV/Vis spectroscopy is commonly called electronic spectroscopy.

In principle, electronic spectroscopy of solid samples may use the same sampling techniques as FTIR spectroscopy. However, photoacoustic and ATR techniques have seldom been used (Kushelevsky and Slifkin 1987). The analysis of paper sheets is usually based on diffuse reflectance measurements. The electronic absorption bands of solid materials are broad and hence detailed analysis of their chromophoric structures is difficult.

Electronic spectroscopy has been widely applied in studies of bleaching and photoyellowing of lignin-rich pulps. Changes in chromophoric structures are best characterized by difference reflectance spectra, which show destruction of coniferaldehyde structures at 350 nm and generation of *o*-quinones at 420 nm (Heitner and Min 1987).

Electronic microspectroscopy has a very good spatial resolution ($\approx$0.1 μm) and it can therefore be applied to the determination of lignin distribution in the cell wall layers by using thin transverse sections of wood (Scott et al. 1969). By this technique, the distribution of specific chromophoric structures, such as coniferaldehydes, in the cell wall has also been determined.

6.3 Spectroscopic Determination of Surface Composition

The applicability of different spectroscopic methods to fiber surfaces is limited by the following factors: (1) fibers are essentially nonconducting, so that methods that analyze emissions of ions or electrons will have to take into account the resulting charging of the surface, (2) deterioration of the surface

by the probe beam, although in some cases inherent in the method of analysis, is complex and may give rise to emissions that are difficult to interpret, and (3) on the scale of the details investigated by different spectroscopic techniques, fibers are rough and heterogeneous, which may make quantitative and/or statistically significant analysis difficult. Thus, only a few of the many surface spectroscopic methods available have been successfully applied to the study of fiber surfaces. These are summarized in Table 6.1. The following discussion will be limited to ESCA (XPS) and SIMS. The use of IR and Raman spectroscopy was considered in Section 6.2.

6.3.1 Electron Spectroscopy for Chemical Analysis (ESCA)

6.3.1.1 Principle of ESCA

In ESCA analysis of solids, the surface is irradiated by well-defined short-wave electromagnetic radiation. By absorbing the radiation, electrons are emitted from the core levels of atoms in the surface layer. Their kinetic energy E_K is given by

$$E_k = E_P - E_B - \phi, \tag{6.4}$$

where $E_P = h\nu$ is the energy of the excitation radiation with frequency ν, E_B is the binding energy of the electron in the atom, and ϕ the so-called "work function", a collective term whose value depends on the sample and the spectrometer. Thus, a series of photoelectron emissions arises that reflect the discrete binding energies of the electrons present in the solid. In ESCA, the

Table 6.1. Spectroscopic methods used for the investigation of cellulosic fiber surface properties

Technique		Description	Information
IR	Infrared spectroscopy	Adsorption of infrared radiation	Surface and bulk chemical structure
Raman	Raman spectroscopy	Adsorption bands in scattered light	Surface and bulk chemical structure
ESCA	Electron spectroscopy for chemical analysis	X-rays eject electrons from atomic levels. Electron energy spectrum is measured	Elementary composition and chemical structure of surface
SIMS	Secondary ion mass spectroscopy	Ionized atoms or fragments of surface molecules are ejected by impact of $\approx$ 1 keV ions and analyzed by mass spectroscopy	Chemical structure of surface

kinetic energies of these emitted electrons are analyzed. Figure 6.9 shows a low resolution ESCA spectrum (a so-called *wide scan*) of an unbleached kraft pulp sample.

Following the pioneering studies by Dorris and Gray (Dorris and Gray 1978a,b; Gray 1978), ESCA has become one of the most important methods for determining the surface composition of papers and fibers. For a comprehensive review, see Istone (1995).

Photoelectron emission is accompanied by secondary processes, as schematically illustrated in Fig. 6.10. The hole created in the core level (Fig. 6.10a) is filled by an electron from a higher level, releasing an amount of energy that can appear as radiation (X-ray fluorescence) or be given up to another electron, which is then emitted (Fig. 6.10b). Such electrons are called Auger electrons. Auger peaks always occur in wide scan ESCA spectra. However, in spectrometers especially designed for AES, the initial emission of electrons is obtained by irradiating the surface with an electron probe beam.

AES is independent of the energy of the incident X-ray radiation. However, since the binding energies in ESCA spectra are always shown as corrected for the energy of the X-ray source, the position of Auger peaks in ESCA spectra will depend on the X-ray source.

Auger peaks have been extensively used in studies of the chemistry of metal surfaces, but applications of the method to fibers or paper are scarce.

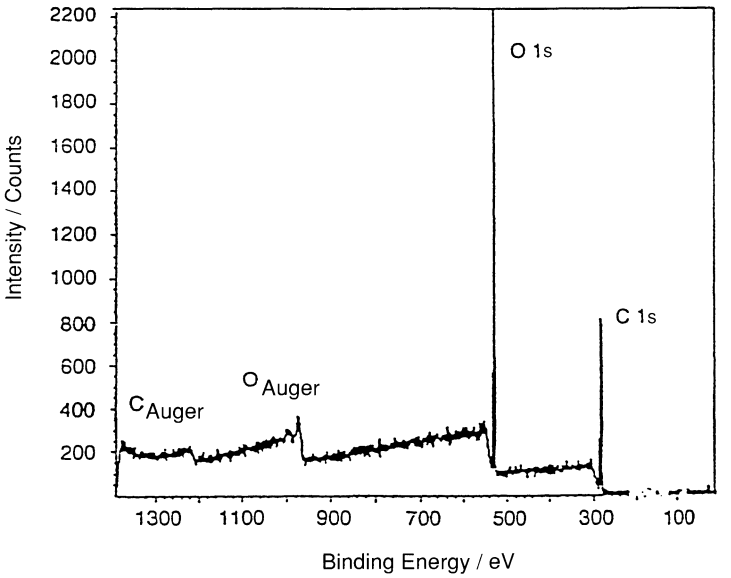

Fig. 6.9. ESCA survey spectrum of softwood lignocellulosic fibers (unbleached kraft pulp), showing C(1s), O(1s), and Auger peaks from oxygen and carbon. The sample was excited by Al Kα radiation. The background "steps" toward low kinetic energy are due to energy loss within the solid. (Laine et al. 1994)

6.3.1.2 The ESCA Spectrometer

The essential features of an ESCA spectrometer are the following:

Radiation Source and Monochromator. X-rays are generated by bombardment of a metallic anode (usually magnesium or aluminum) with electrons of sufficient energy. Monochromatization depends on the diffraction of X-rays in a crystal. For routine analysis, it is not absolutely essential to use monochromatic X-rays, but monochromatization strongly enhances the energy resolution of ESCA analysis and reduces risks for sample deterioration by minimizing the radiation dosage.

Vacuum System. Electrons traveling from the sample surface to the detector should encounter as few gas molecules as possible, and contamination by adsorbed gas molecules must be minimized. This makes it necessary to work at ultra high vacuum (pressure $\leq 2 \times 10^{-4}$ Pa).

Samples. The most important restrictions on samples are that they should not be deteriorated by X-ray irradiation, and that they should resist ultrahigh vacuum without significant modification. The vacuum restriction rules out the study of wet or humid samples unless they are cooled to very low temperatures, but otherwise a very wide range of different samples can be investigated. Due to the extreme surface sensitivity of ESCA, it is very important to avoid contamination during sample preparation and handling (for example, the sample surfaces must never be touched by skin). Sample size is usually limited to a few square centimeters and precise manipulation of

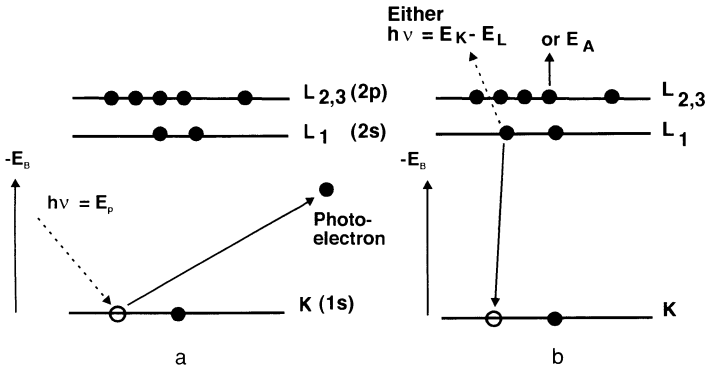

Fig. 6.10. The ESCA and Auger electron emissions. **a** When radiation (of energy E_p) is absorbed, a photoelectron is emitted from the core level of the atom (ESCA emission). The kinetic energy of the electron is $E_P - E_K$. For this to occur efficiently, E_P should be $\geq 5E_K$. **b** the hole in the K shell is filled by an electron from L_1. The released energy, $E_K - E_L$, can appear as a photon (X-ray fluorescence) or can be given up to another electron, which is ejected (Auger emission)

the sample should be possible without bringing the analysis chamber to atmospheric pressure.

Usually the width of the analyzed area is of the order of millimeters. Recent developments of the focusing systems have made it possible to reduce the minimum width to a few tens of micrometers or even less. It is also possible to record two-dimensional images of the distribution of a particular element, or even of an element in a particular chemical state. The implications for future studies of even single fibers are obvious. A matter that should also be taken into account is the stability of surfaces when they are exposed to X-ray beams. Wood polymers seem to be reasonably stable during the usual exposure times in ESCA analysis.

Electron Energy Analyzer (Electron Spectrometer). All commercial spectrometers use an analyzing system that retards the electrons that enter the actual analyzer to a fixed energy. Because focusing depends on how much the electrons are retarded, the analyzed area changes with the kinetic energy of the emitted electrons, which should be accounted for in quantitative analysis. In some recent commercial spectrometers the focusing has been designed so that this problem is rendered largely negligible.

6.3.1.3 Escape Depth

The photoelectrons interact strongly with the atoms in the solid material. Hence, the intensity of escaping electrons decreases rapidly as the depth of analysis increases. The *escape depth* (also called the inelastic mean free path) of electrons λ, is the thickness of material required to reduce an initial intensity n_0 of electrons to n_0/e. λ is typically of the order of 1–3 nm. Accordingly, because the intensity decreases roughly exponentially with thickness, $\approx$95% of the electrons detected are emitted from a depth $<3\lambda$. Thus, ESCA analysis is confined to 3–7 atomic layers in the surface. The surface sensitivity of ESCA can be further enhanced by analyzing electrons emitted at different angles to the surface (see below).

6.3.1.4 Qualitative Elemental Analysis

The surface yielding the spectrum in Fig. 6.9 contains cellulose, hemicelluloses, lignin, and low-molecular weight hydrophobic compounds (extractives). Peaks due to the emission of electrons from the core levels (1s) of oxygen and carbon occur on a background that generally increases toward low kinetic energy (high binding energy). Two peaks are due to Auger electrons emitted from O and C. Full survey spectra often also show peaks due to emissions from valence levels at the low binding energy end (see below).

6.3.1.5 Quantitative Analysis

Simple quantitative analysis of a solid surface is based on the assumption that in a homogeneous sample the intensity n_{ik} of electrons emitted from the k shell of an element i is proportional to the mole fraction of this element

$$x_i = \frac{n_{ik}}{n_{ik}^0}, \tag{6.5}$$

where n_{ik}^0 is the intensity from pure i. The intensity n_{ik} is given by

$$n_{ik} = I_0 N_i \sigma_{ik} \lambda_{ik} T_{ik}, \tag{6.6}$$

where I_0 is the intensity of the incident radiation, N_i the number concentration of i-atoms in the surface, σ_{ik} the photoemission cross section (PCS) of the electrons in the ik shell, λ_{ik} their escape depth, and T_{ik} a factor that depends on the construction of the spectrometer. For many instruments, T_{ik} is inversely proportional to the kinetic energy E_{ik}. On the other hand, $\lambda_{ik} \approx \text{const} \cdot (E_{ik})^a$, where a for many organic materials varies between 0.7 and 1.0, so that the product $T_{ik}\lambda_{ik}$ varies relatively slowly with E_{ik}. For this reason, a semi-quantitative determination of the composition of the surface can be made by simply comparing the intensities of spectral peaks, provided that the PCSs are known. However, the accuracy of absolute surface concentrations determined from ESCA of homogeneous samples cannot generally be considered better than about 5%. Theoretical PCSs have been calculated for a great number of atomic orbitals. A more common practice, however, is to use empirically derived factors. Tabulations can be found, for example, in Briggs and Seah (1990).

Depending on the element, the smallest amount that can be detected by ESCA varies between 0.05 and 1 atomic %.

6.3.1.6 Chemical Shifts

One of the most important features of ESCA is that the bonding energy of electrons emitted from a given core level in a given element increases when the density of electrons on the atom decreases. This dependence can be due to differences in the molecular environment, in oxidation states, in location in a crystal lattice, etc. It gives rise to *chemical shifts* in the spectra.

Figure 6.11 shows a high-resolution spectrum of the C(1s) peak of fibers at different degrees of delignification. Four distinct peaks can be resolved, corresponding to the four chemical environments of carbon given in Table 6.2. These are, indeed, expected to occur in lignocellulosic fibers. The C_1 peak will be mainly due to lignin and extractives, the C_2 and C_3 peaks to lignin, cellulose and hemicelluloses, and the C_4 to carboxyl groups.

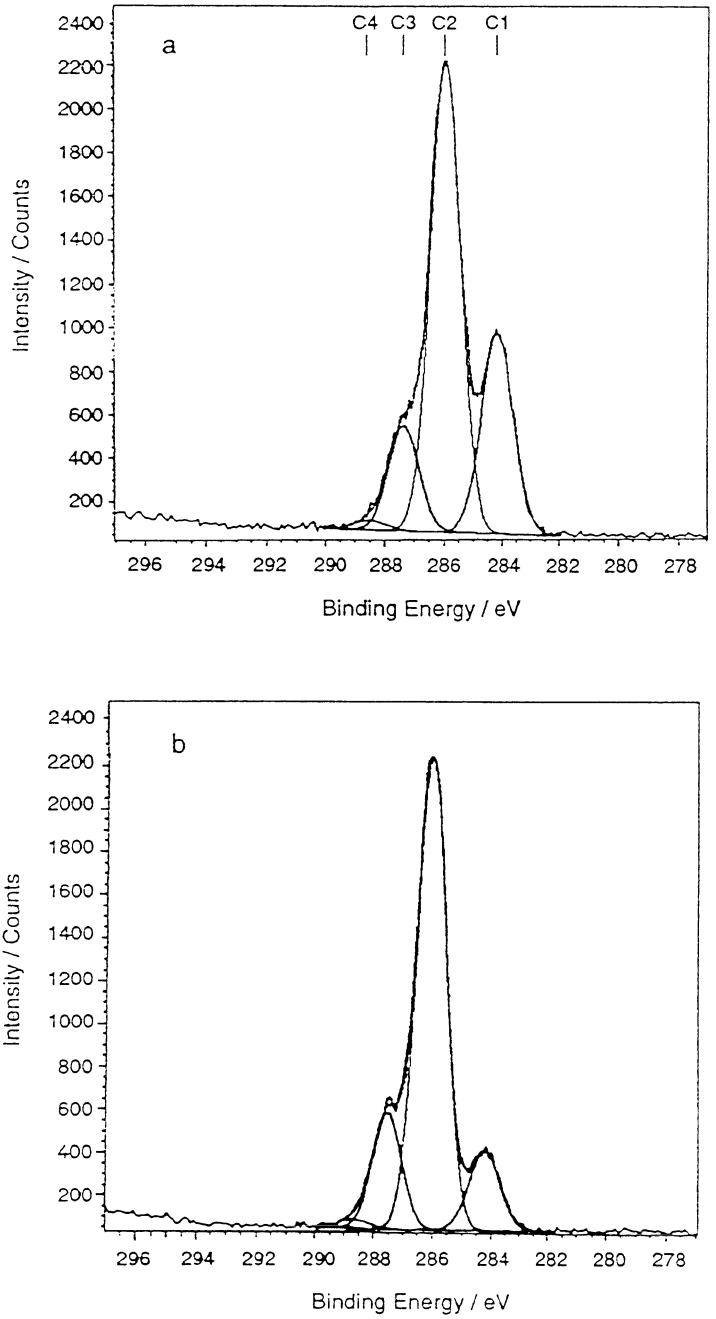

Fig. 6.11. High-resolution spectrum of the C(1s) peak in the ESCA spectrum of unbleached kraft pulp at different degrees of delignification. *C1* carbon bound to hydrogen or carbon atoms only, *C2* carbon with one bond to oxygen, *C3* carbon with a double bond to oxygen or with single bonds to two oxygen atoms, and *C4* carbon in carboxyl groups. **a** Fibers containing 8.9% lignin; **b** fibers containing 2.8% lignin. Note the much smaller fraction of the *C1* carbon in the surface of the latter fibers. (Laine et al. 1994)

Table 6.2. Some chemical shifts in ESCA spectra of use in the analysis of fibers. (Briggs and Seah 1990; Briggs and Beamson 1992)

Core level	Chemical bonds	Binding energy (eV)	Chemical shift[a] (eV)
C *1s*	To carbon or hydrogen only (C1)	285.0	
	One bond to oxygen (C2)		1.5
	Two bonds to oxygen (O-C-O, C = O) (C3)		3.0
	Carboxylic group $-CO_2H$ (C4)		4.0–5.0
S *2p1/2*	$R\text{-}SO_3H$	169	
	$R\text{-}SO_2\text{-}R$		−2.5
O *1s*	Different functionalities	533	±2
N *1s*	$-N(CH_3)_2$, $-NH_2$, $-C{\equiv}N$	399–401	
	$-NH_3^+$, $-N(CH_3)_3^+$		1.5
	$-NO_2$		7

[a] Relative to the energy given in the column to the left.

From the wide scan and the detailed spectrum of the C(1s) peak, the O/C ratio and the relative amounts of the different carbons in the surface can be readily calculated. Because these amounts are different in the different pulp components (for example, the amount of C_1 carbon decreases in the order extractives > lignin > carbohydrates), the amounts of lignin and extractives in the surface can be estimated by analyzing spectra recorded before and after extraction. Typical spectra are shown in Fig. 6.11. The surface coverage of extractives can be calculated from the following equation:

$$\phi_{\text{extr}} = \frac{\text{O/C(before extraction)} - \text{O/C(after extraction)}}{\text{O/C(extractives)} - \text{O/C(after extraction)}}, \tag{6.7}$$

where O/C (extractives) is calculated from the spectrum of pure model extractive compounds. Similarly, assuming that the thickness of the regions where lignin is located, is greater than $\approx 3\lambda$, the following equation gives the lignin surface coverage:

$$\phi_{\text{lignin}} = \frac{\text{O/C(pulp sample)} - \text{O/C(pure pulp)}}{\text{O/C(lignin)} - \text{O/C(pure pulp)}}, \tag{6.8}$$

where O/C(pulp sample) is the O/C ratio of the sample after extraction and O/C(lignin) is the ratio of a pure lignin model compound. A value for O/C(pure pulp) is obtained from extracted and fully bleached fibers, which contain practically no lignin. Table 6.3 gives O/C ratios for model compounds and bleached softwood kraft pulp.

Table 6.3. The oxygen/carbon atomic ratio (O/C) and the relative amounts of different carbons (C 1s) of model compounds and components in softwood fibers (unbleached kraft pulp). C1 = carbon bound to hydrogen or carbon atoms only, C2 = carbon with one bond to oxygen, C3 = carbon with a double bond to oxygen or with single bonds to two oxygen atoms, and C4 = carbon in carboxyl groups. (Dorris and Gray 1978a,b; Laine et al. 1994)

Model compound	O/C	C1	C2	C3	C4
Cellulose (theoretical)	0.83	–	83	17	–
Bleached Kraft pulp	0.80	6	75	18	1
Arabinoglucuronoxylan (theoretical)	0.81	–	78	19	3
Xylan (from pulp)	0.83	5	67	24	4
Lignin (theoretical)	0.33	49	49	2	–
Kraft lignin (from pulp)	0.32	52	38	7	3
Oleic acid (theoretical)	0.11	94	–	–	6
Extractives (from pulp)	0.12	93	5	–	2

6.3.1.7 Angular Dependent Effects

In many cases, for example in investigations of adsorbed polymer layers, it is of great interest to determine the composition in the direction perpendicular to the surface. Such information can be derived by increasing the surface sensitivity through variation of the angle of analysis.

The principle of the method is shown in Fig. 6.12. If the take-off angle is θ, the vertical depth of analysis is given by

$$d = 3\lambda sin\theta, \tag{6.9}$$

i.e., if $\theta = 90°$, the depth of analysis $d \approx 3\lambda$. A commonly used model in surface analysis is that a thin, uniform surface layer (f) of thickness d containing the element i covers an infinitely thick substrate (s) containing an element j. It can be shown that, provided the kinetic energies of the electrons from layers s and f are not too different, the ratio of the intensity from the surface layer n_f to that from the substrate n_s is given by

$$r = \frac{n_f}{n_s} = R\left[exp\left(\frac{d}{\lambda_{i,f}\, sin\theta}\right) - 1\right], \tag{6.10}$$

where λ_{if} is the escape depth of the electrons in the surface layer and R is a constant containing the PCSs and number concentrations of the atoms in the surface layer and in the substrate. r can be determined directly by measuring the intensities of the two peaks as a function of the scattering angle θ.

In practice, more complex expressions are often required because surface roughness and system geometry introduce factors that are also dependent on θ, and because λ may be different for the substrate and the overlayer. Nevertheless, very useful information has been derived by this type of analysis. As

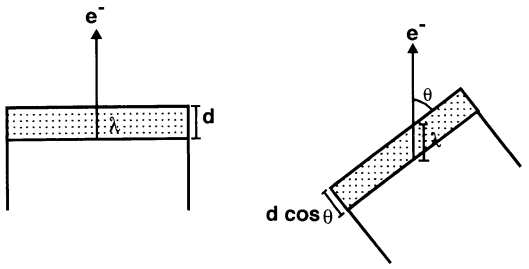

Fig. 6.12. Angular dependent ESCA. λ is the escape depth of the electrons. When the angle θ between the path of the electron to the detector and the surface of the sample decreases, the depth of analysis decreases by the factor $\cos\theta$

an example, the angular dependence of the N(1s) peak of a typical cationic retention polymer, polyethyleneimine (PEI) adsorbed on sulfated polyethylene (PE) is shown in Fig. 6.13. For the layer adsorbed at pH 4.0, $r = n(\mathrm{NH})/n(\mathrm{NH_2^+})$ is lower and does not depend on the exit angle, while after adsorption at pH 9.0, r is higher and increases when the exit angle decreases. A straightforward interpretation of these results is that the configuration of PEI when adsorbed at pH 4.0 is essentially flat on the surface while adsorption at pH 9.0 gives a "layered" configuration with the charged ($>\mathrm{NH_2^+}$) groups close to the surface and the neutral groups further out.

6.3.1.8 Derivatization of Functional Groups

The lack of specificity in the chemical shifts of different functional groups often causes problems when more complex surfaces are to be analyzed. One way to overcome this problem is to use specific reagents that react with only one type of functional group and contain an atom or atoms that can be easily identified. The advantage of this type of analysis is that it becomes possible to analyze surface groups that, before derivatization, have the same chemical shift. It also becomes possible to determine the concentration of different functional groups. Further, if the reagents contain atoms with high photoemission cross sections, the sensitivity of the analysis is enhanced. A severe problem is that the surface may be so strongly modified that it no longer gives information on the conditions of the original surface. Reagents that are effective in the gas phase have alleviated this problem. An extensive table of possible reactions can be found in Briggs and Seah (1990).

6.3.1.9 Effects of Charging

During an ESCA analysis, the emission of photoelectrons generates a positive charge onto the surfaces of insulating materials, including cellulose and paper.

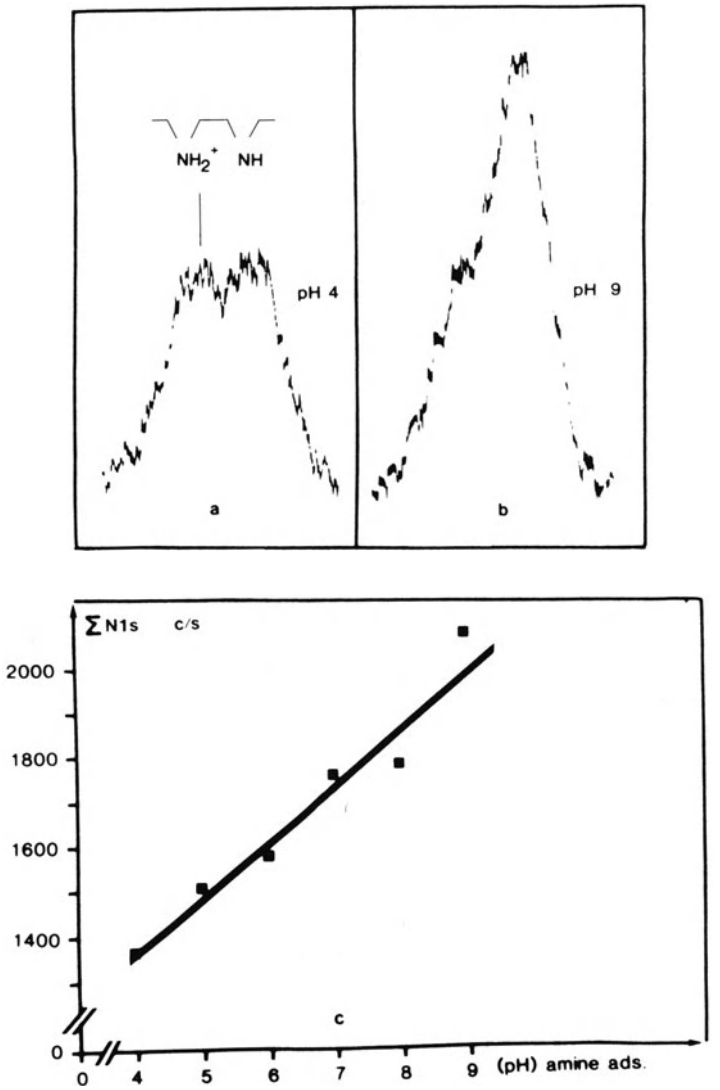

Fig. 6.13. **a** The N(1s) ESCA peaks (>NH and $>NH_2^+$) of polyethyleneimine adsorbed on sulfated polyethylene at pH 4.0 and **b** at pH 9.0. **c** The angular dependence of the $n(NH)/n(NH_2^+)$ peak height ratio on the exit angle of the electrons for PEI adsorbed at pH 4 and 9. (Larsson et al. 1982)

This will give rise to problems because of the shift of the spectrum on the energy scale.

Several methods have been used to correct for these shifts. These methods include partial covering of the sample with materials with precisely known binding energies, mixing of samples with powders that contain a component of a known binding energy, and using the binding energy of chosen chemical

groups as an internal standard. The last method is probably the best and widely used in studies of polymers.

The charging of insulators is stabilized by subjecting the sample to free electrons generated by an electron flood gun or a hot wire. The methods have been developed to a level where excellent stability is achieved.

6.3.1.10 Valence Band Spectroscopy

Photoelectrons may also be emitted from outer shells, i.e., from bonding orbitals. The spectra of such electrons are referred to as valence band or molecular orbital spectra. Valence bond electrons are much more sensitive to changes in chemical structures than are core electrons, and the spectra may be used for detailed identification of surface structures. However, the probability of emission of valence bond electrons is much less than that of core electrons, resulting in poor sensitivity, so that long acquisition times are required. This may result in severe sample damage. Therefore, routine recording of reliable valence band spectra of organic surfaces was not possible until the relatively recent development of monochromatic X-ray sources and better detectors. So far, there is little information in the literature on valence band spectra from lignocellulosic materials. Some valence band energies of interest in fiber analysis are given in Table 6.4.

6.3.2 Secondary Ion Mass Spectroscopy (SIMS)

6.3.2.1 Principle of SIMS

Secondary ion mass spectroscopy (SIMS) is the most sensitive of all the surface analysis techniques. It is one of the several desorption ionization (DI) mass spectroscopic methods (Fig. 6.14). Other types of DI techniques include fast atom bombardment mass spectrometry (FAB/MS), which uses neutral atoms to sputter fragments from surfaces, and matrix assisted laser microprobe or laser desorption mass analysis of ions (MALDI).

In *static* SIMS, the surface is bombarded with low energy (100 eV to 10 keV), low flux density ion beams. During the time required to measure a mass spectrum, less than a monolayer is sputtered off the surface of the material. It then becomes possible to determine both the chemical composition and the distribution of elements in the outermost surface of the material.

In *dynamic* SIMS, higher primary ion energies and densities are used. This increases the sensitivity of the analysis, but the surface is eroded. Dynamic SIMS can be used for trace elemental analysis (concentrations in the ppb range) and depth profiling studies. Table 6.5 summarizes some of the features of SIMS analysis.

Table 6.4. Valence band energies in ESCA spectra. (Conners and Banerjee 1995; Briggs and Beamson 1992)

Element	Orbital	Binding energy range (eV)
Carbon	2p	4–12
	2s	13–22
Nitrogen	2s	21–25
Oxygen	2s	26–30
Sodium	2p	30–32
Chlorine	3p	3–6
	3s	14–15

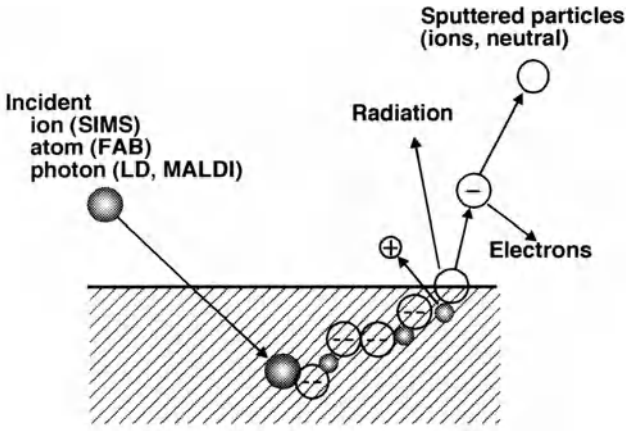

Fig. 6.14. Schematic illustration of the different ion desorption techniques. *SIMS* secondary ion mass spectroscopy; *FAB* fast atom bombardment; *LD* laser desorption; *MALDI* matrix assisted laser desorption ion spectroscopy

Table 6.5. SIMS as a method for surface analysis of organic materials

Advantages	Extremely surface sensitive (0.2–20 nm)
	Can be used in both destructive and nondestructive mode
	Composition profiling in the z direction and scanning in the xy plane is possible
	Lateral resolution 1–3 μm
	Very high sensitivity elemental analysis
	Determination of detailed chemical structure (static SIMS)
Disadvantages	Very difficult to quantify
	Requires ultrahigh vacuum
	Very expensive equipment
	Relatively slow and expensive analysis
	Charging of surface causes problems

6.3.2.2 Instrumentation

In a SIMS spectrometer, an ion beam is focused onto a solid-phase sample. This results in the sputtering of secondary species (ions, neutral atoms, molecular fragments, and electrons) from the topmost layer of the surface. The emitted ionic species are analyzed by a mass spectrometer, giving a positive or negative ion SIMS mass spectrum. Ions emitted from the sample can also be collected to form SIMS images of their spatial distribution on the sample surface in the *xy* plane.

The *primary ion source* provides a stable energetic beam of ions created by discharge through a gas (argon and oxygen), field ionization of liquid metals (gallium ions are often used), and thermal ionization of alkali metals (usually cesium).

The *mass analyzer* may be of several different types. In most SIMS instruments, quadrupole or sector mass analyzers are used. These are generally limited to ions with masses <2000. Recently, the use of time-of-flight (ToF) mass spectrometers has become more common. This greatly improves sensitivity, range of mass detection, and lateral resolution, and enables accurate analysis of complex species such as lignocellulosic materials.

As in ESCA, it is essential that ultrahigh vacuum be maintained in the analyzing chamber, so that the analysis is not disturbed by contaminants and collisions of the sputtered ions with other molecules. The restrictions on samples are quite similar to those of ESCA.

6.3.2.3 The Sputtering and Ionization Processes

Detailed theoretical models of the sputtering process have been developed, but only the most important features of this technique are discussed in the following.

Both theory and experiment show that the sputtered ions for any individual primary ion originate from the top 2–3 nm of the surface. The reason for this is the very fast dynamics of interaction of the incident particles with the molecules in the surface. The interaction results in rapid sequential collisions (a cascade) within a very small volume, which creates secondary ions with sufficient velocity to be able to leave the surface.

When the flux of primary ions is low, the ionization process is not very fast and it may take several minutes or even hours to erode a monolayer. This is utilized in the so-called static SIMS mode.

If the flux of primary ions is very high, many secondary ions leave the surface in a very short period, and the surface erodes at a rate of a monolayer in a few seconds (dynamic SIMS). This rate of decomposition can be controlled so that depth profiling becomes possible.

Interpretation of SIMS spectra (of positive or negative ions) may be complex, as is that of ordinary mass spectra of organic ions. The principles of interpretation have been well described by Briggs et al. (1989, 1992).

6.3.2.4 Applications

The use of SIMS to gain a better understanding of the bulk and surface chemistry of cellulose fibers and paper sheets has been well reviewed by Detter-Hoskin and Busch (1995). Among these applications may be mentioned the analysis of sizes present in paper surfaces (Brinen et al. 1991; Brinen and Proverb 1993; Kulick and Brinen 1996; Fig. 6.15), the spatial distribution of organochlorine in bleached pulp (Tan and Reeve 1992; Fig. 6.16), and the distribution of inorganic ions in sheets of bleached groundwood fibers (Saastamoinen et al. 1994).

6.4 Surface Analysis by Enzyme Treatment

Specific xylan and glucomannan degrading enzymes can be used as tools for characterization of cellulosic fibers. Thus, purified *Trichoderma reesei* xylanase and mannanase can be used for selective solubilization of xylan and glucomannan from pulps. The chemical composition of the solubilized fraction can then be determined by HPLC (Buchert et al. 1993; Tenkanen et al. 1995). Due to their relative large molecular weight, the enzymes are expected to primarily hydrolyze polysaccharides in the most accessible parts of the fibers, i.e., the fiber surface. By combining this enzymatic solubilization with pore size measurements and titration, the location of the hemicelluloses can be evaluated.

The degree of solubilization can be controlled by enzyme dosage and hydrolysis time. In extensive xylanase or mannanase treatments, up to 40% of the corresponding hemicelluloses can be selectively solubilized from kraft pulps (Table 6.6). However, the degree of enzymatic solubilization depends on the pulp type.

For example, enzymatic solubilization of pulp xylan combined with potentiometric titration and polyelectrolyte adsorption has been used in the analysis of hexenuronic acids in kraft pulps (Buchert et al. 1995; Laine et al. 1996). It was found that in birch kraft pulp the most accessible xylan, i.e., xylan solubilized in xylanase treatment, contained a larger number of acid groups than the residual xylan, whereas for pine kraft pulp the situation was the reverse (Table 6.7). It appears that in both birch and pine kraft pulps, the amount of uronic acid sidegroups in xylan depends on the location of xylan in the fibers.

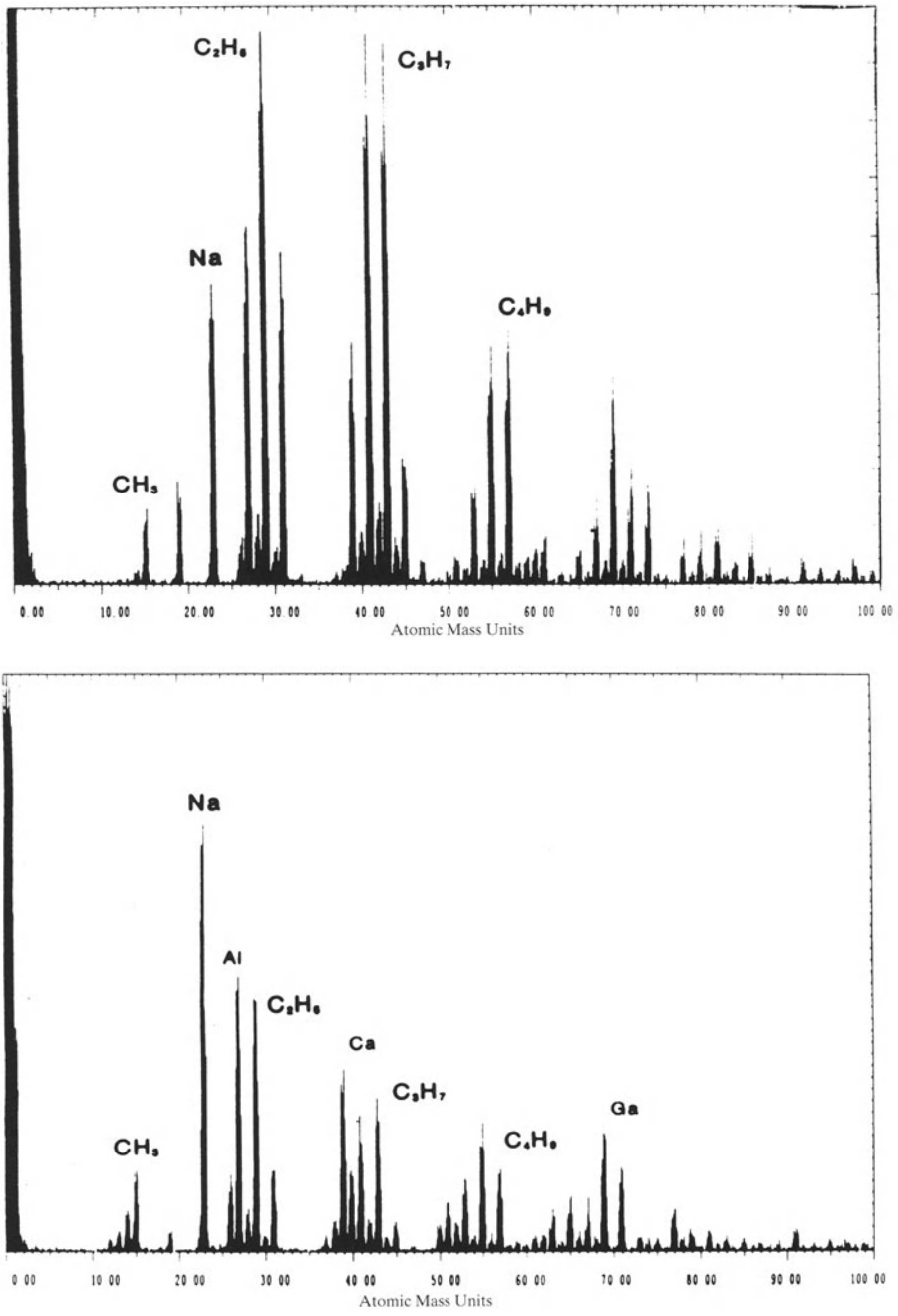

Fig. 6.15. *Top*: Static SIMS spectrum of a wet strength paper using xenon ion excitation. *Bottom*: SIMS spectrum of a wet strength paper using gallium ion excitation after most of the hydrocarbon surface has been removed by ion beam erosion. (Brinen et al. 1991)

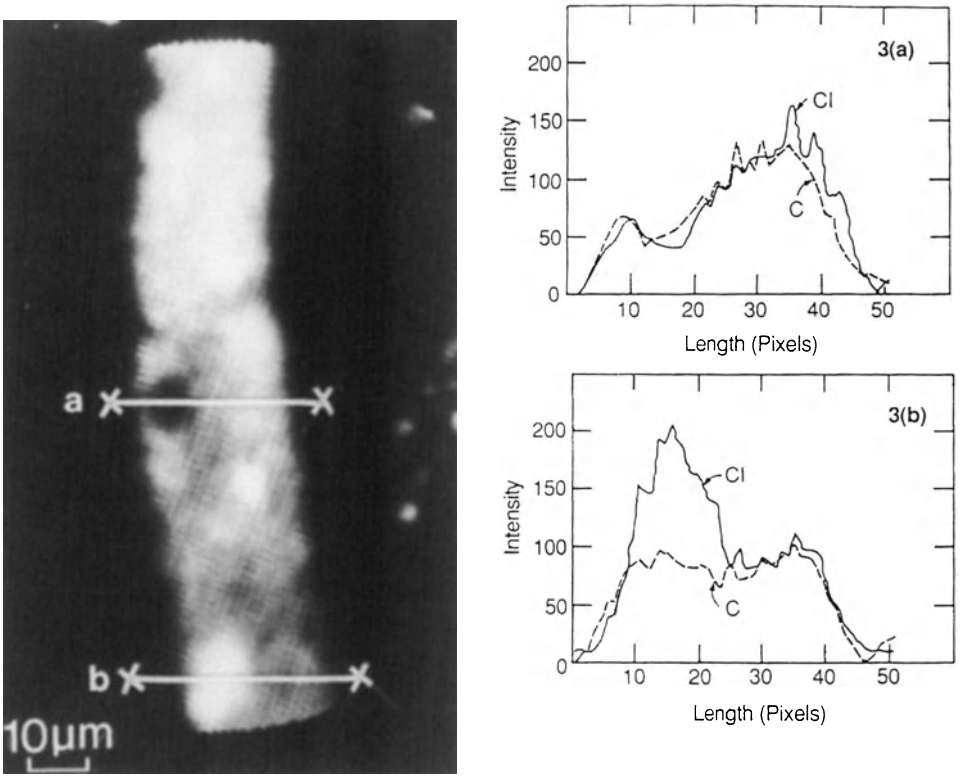

Fig. 6.16. *Left* ^{35}Cl SIMS image of a flattened softwood kraft fiber bleached with the sequence O(DC)(E + O)HD and soxhlet extracted with 1:1 ethanol/toluene (1:1) for 8 h. Traverses (*right*, ^{35}Cl peak intensity) from left to right are shown in the adjacent line-scane plot. (Tan and Reeve 1992)

Table 6.6. Solubilization of hemicellulose components by *Trichoderma reesei* xylanase and mannanase treatments from different pulp types. (Buchert et al. 1993)

Pulp	Enzyme	Treatment	Solubilization		
			Percent of dry wt.	Percent of xylan	Percent of glucomannan
Pine kraft	Xylanase	Limited	0.8	8.0	
		Extensive	3.7	32.1	
	Mannanase	Limited	0.4		5.5
		Extensive	2.0		27.8
Birch kraft	Xylanase	Limited	2.2	9.2	
		Extensive	10.7	37.0	
TMP	Xylanase	Extensive	0.7	nd[a]	

[a] nd = not determined.

Table 6.7. Calculated molar ratios of xylose (Xyl):uronic acids (CO_2H) in different xylans. (Laine et al. 1996)

Sample	Pine Xyl:COOH (mol/mol)	Birch Xyl:COOH (mol/mol)
Wood xylan (Sjöström 1993)	5:1	10:1
Total pulp xylan	12:1	20:1
Residual xylan in pulp after xylanase treatment	12:1	21:1
Solubilized xylan in xylanase treatment	14:1	12:1

6.5 Determination of Functional Groups by Specific Chemical Reactions

Besides the spectroscopic methods (NMR, FTIR, Raman, UV) and pyrolysis, certain chemical structures of wood and pulp can be analyzed by simple wet chemical methods. In principle these methods include a selective reaction of a certain functional group of the solid sample and subsequent analysis of either the reactant consumed or the soluble product formed. In most cases, the sample preserves its fiber structure.

Quantitative carbohydrate and lignin analysis is described in Chapters 4 and 5, respectively. It is important to note that neither permanganate nor chlorine are completely selective for lignin but react with all unsaturated structures, such as 4-deoxy-β-L-*threo*-hex-4-enuronic acid groups of kraft pulps (Vuorinen et al. 1996).

The *methoxyl* content of wood and pulp samples has been of interest for a long time because the methoxyl content can be correlated with the amount and structure of lignin or xylan depending on the type of the sample. The traditional method is to cleave the *O*-methyl groups by nucleophilic substitution by iodide and to determine the methyl iodide liberated by titration or GC. The *O-methyl groups in phenolic guaiacyl (and syringyl) structures* are liberated as methanol when treated with periodate (Lai et al. 1990). The amount of the liberated methanol can be quantified with GC.

Quantitative wet chemical analysis of *phenolic groups* in wood and pulp samples include an *O*-acetylation step and subsequent selective aminolysis of the aryl acetates with pyrrolidine (Gellerstedt and Lindfors 1984). *N*-acetylpyrrolidine is formed as the product which is determined by GC. Pyrrolidine also deacetylates acetyl glycosides and therefore an initial reduction of the free aldehyde end groups of polysaccharides can be necessary.

The *O-acetyl groups* of hardwood xylans and softwood galactoglucomannans can be analyzed by a simple hydrolysis and subsequent GC or HPLC of acetic acid.

Carbonyl groups are present in native wood both in lignin and in reducing end groups of cellulose and hemicelluloses. Most of these are destroyed during kraft pulping but certain bleaching chemicals, most notably ozone, generates additional carbonyl groups both in lignin and polysaccharides. The carbonyl groups in nonreducing glucose units of cellulose are especially harmful as they may induce depolymerization of the polymer chain during subsequent alkaline bleaching stages. FTIR spectroscopy is sensitive for carbonyl groups, but their tendency to form hemiacetals and hydrates generally prevents a quantitative analysis. The methods applied for determination of carbonyl groups in pulp samples are generally based on oximation. The degree of oximation is determined e.g. from the nitrogen content in elemental analysis (Kjeldahl determination or ESCA).

The *4-deoxy-β-L-threo-hex-4-enuronic acid groups* of kraft pulps are hydrolyzed under mild conditions, giving 2-furancarboxylic and 5-formyl-2-furancarboxylic acids as the products (Vuorinen et al. 1996). Both of these have a strong UV absorption (at 245 and 280 nm, respectively) which enables their quantification simply by UV spectrophotometry.

In most of the wet-chemistry methods it is necessary to follow the consumption of the reagent or the formation of the product as the function of time because competing side reactions usually occur and it is necessary to make a background correction for them. As a result, the wet chemical methods are often laborious.

6.6 Determination of Ionizable Groups (Fiber Charge)

Unbleached cellulosic fibers carry a negative charge because of the presence of ionizable groups bound to the polymeric backbone or to extractive compounds (rosin and fatty acids). As a result of reactions and dissolution during pulping and bleaching, a part of the groups are removed, but new groups can be formed as well (Chap. 1). The ionic groups on cellulosic fibers are of great importance for the swelling of the fibers and the strength and optical properties of paper (Eriksson and Sjöström 1968; Sjöström and Eriksson 1968; Lindström and Carlsson 1982a,b; Engstrand et al. 1991; Laine and Stenius 1997).

The fiber charge can be determined by ion exchange and titration or, more indirectly, by measurement of the electrokinetic potential (zeta potential). The interpretation of electrokinetic measurements on fibers in terms of charge densities is complex and has to be based on rather drastically simplifying assumptions. Electrokinetic methods will therefore not be considered here.

In wood and chemical pulps, the carboxylic acid groups are the main carriers of charge (Sjöström 1989). In aqueous fiber suspension, the uronic acid (carboxylic) groups (pK 3–4) begin to dissociate appreciably above pH 2–3.

The phenolic hydroxyl groups (in lignin) are much weaker acids and alkaline conditions (pH > 9) are needed for their ionization. The different types of alcoholic groups are such weak acids that they are ionized only in strongly alkaline solutions.

The sulfonic acid groups present in the residual sulfite pulp lignin must also be considered. Indeed, in the case of some high yield pulps, including CTMP, they are the dominant type of acid groups.

6.6.1 Ion Exchange

Much research has been devoted to the determination of carboxylic acid groups in cellulosic fibers, including different types of chemical pulps. The basic ion exchange methods still in use were developed some decades ago (for literature, see, e.g., Sjöström and Haglund (1961) and the references therein). In principle, the total ion exchange capacity of the fibers, equivalent to the total number of carboxylic acid groups, is measured. The most usual method is based on hydrogen ion exchange. The fiber sample is first treated (preferably in a small glass column) with an excess of acid (usually 0.1 M HCl is used) to convert the carboxylate groups to hydrogen ion form. The fibers are then thoroughly washed with deionized water to remove excess free acid. Finally, an excess of salt (sodium chloride solution) is added and the hydrogen ions (HCl) liberated in the exchange of H^+ for Na^+ are titrated potentiometrically or simply using an indicator. One source of errors in this method is the tendency of certain carboxylic acid groups to be lactonized during the acid treatment.

In addition to the procedure described above, alternative ion exchange methods are available in which divalent salts are used. One benefit of these is that lactonization is avoided and the sensitivity is increased. One convenient and reliable method is based on magnesium ion exchange. The sample is introduced into a glass column and treated with 0.1 M magnesium chloride solution. After saturation, the excess of free magnesium chloride is thoroughly washed out with deionized water. Finally, the bound magnesium ions are eluted with acid and measured by complexometric titration with EDTA. Because of the high detection sensitivity, very low concentrations of magnesium ions can be titrated with high precision. For details, see Sjöström and Haglund (1961).

Finally, it should be mentioned that organic bases (dyes or compounds having a strong UV absorption, e.g., methylene blue) have also been used for quantitative determination of carboxylic acid groups in cellulosic fibers. A serious source of errors associated with these methods is the tendency of dyes to bind not only through ion exchange but also by van der Waals interactions.

In the case of various types of unbleached sulfite pulps, the measurements of ion exchange capacity by the methods described above give the total amount

of carboxylic and sulfonic acid groups. Of the few methods available for the specific determination of sulfonic groups in the presence of carboxylic groups, a procedure in which benzidine hydrochloride is used as reagent has been found to be both simple and reliable (Sjöström and Enström 1966). However, the use of benzidine hydrochloride has been discouraged or banned because it is classified as a carcinogenic chemical. As an alternative, a similar method, in which quinoline hydrochloride is used instead of benzidine hydrochloride, has been published (Westermark and Samuelsson 1993).

The number of sulfonic acid groups could also be estimated on the basis of sulfur analyses, but it must be taken into account that not necessarily all of the sulfur in fibers occurs in the form of sulfonic acid groups.

6.6.2 Conductometric Titration

Direct conductometric titration of cellulosic fibers was suggested a long time ago but a full theoretical discussion of this method was not given until about a decade ago (Katz et al. 1984; Scallan et al. 1988). Failure to realize the importance of using a constant background electrolyte concentration during the titration has led to undue criticism of the possibility of applying the method to the separate determination of acid groups of different strength, especially sulfonic and carboxylic acid groups in sulfite pulp fibers.

Experimentally, the conductometric titration of fibers is similar to that of soluble acids. The total specific conductivity of the solution, κ, is given by the sum

$$\kappa = \sum_i [X_i]\Lambda_i, \tag{6.11}$$

where $[X_i]$ is the concentration of ionic species i with molar conductivity Λ_i. Because hydrogen and hydroxyl ions conduct electricity much better than other ions, the conductivity will decrease or increase markedly when the concentration of these ions decreases or increases. Molar conductivities are known for a great number of commonly occurring ions, so that theoretical titration curves can be easily calculated, provided that the ionic concentrations are known. It is essential to realize that in this calculation it is necessary to account for the nonspecific distribution of ions into the fibers. At a low background electrolyte concentration the result of this distribution is that it is difficult to observe sharp equivalence points in conductometric and potentiometric titrations.

In fiber suspensions, the acid groups are confined to the porous structure in the fiber wall. On the other hand, low-molecular weight ions (hydrogen, hydroxyl, and background electrolyte ions) in the system are freely mobile and tend to distribute themselves over the whole suspension. Therefore, upon dissociation of the acid groups, an electrostatic potential, the *Donnan potential*, is created between the fiber phase and the external solution. This potential

increases with increasing degree of dissociation of the acids and tends to keep the hydrogen ions confined to the fiber phase. Hence, the acids appear to become weaker at high degrees of dissociation. Their apparent dissociation constants $K_{app,i}$ are given by

$$K_{app,i} = K_i \, exp\left(-\frac{F\psi}{RT}\right) = \frac{[B_i]\{H\}}{[HB_i]} exp\left(-\frac{F\psi}{RT}\right), \tag{6.12}$$

where K_i is the intrinsic dissociation constant, ψ is the Donnan potential, {H} is the hydrogen ion activity, $[B_i]$ and $[HB_i]$ are the concentrations of dissociated and undissociated acid HB_i, F is the Faraday constant, and R and T have their usual meaning. The total degree of dissociation Z is defined by

$$Z = \frac{\sum_i [B_i]}{C_B} = \frac{\sum_i K_i [HB_i]\{H\}}{C_B}, \tag{6.13}$$

where C_B is the total concentration of acid groups bound in the fibers. The summation is taken over all acids bound to the fibers.

The distribution of any freely mobile ion X_j with charge z_j between the external solution (E) and the fiber phase (D),

$$X_{j,E} \leftrightharpoons X_{j,D} \tag{6.14}$$

is determined by the Donnan potential only. The distribution can be calculated from the distribution constant

$$\lambda = exp(-z_i \psi F/RT) = \frac{[X_j]_D}{[X_j]_E}, \tag{6.15}$$

and the mass and charge balance equations

$$[X_j]_E + [X_j]_D = C_j, \text{ and} \tag{6.16}$$

$$\sum_j z_j [X_j]_D + ZC_B + \sum_j z_j [X_j]_E = 0, \tag{6.17}$$

where C_j is the total concentration of ion j. When the concentration of background electrolyte is very low, the Donnan potential is high and the hydrogen ions will move strongly into the fiber phase. Accordingly, the conductivity of the external solution will be low and it becomes difficult to identify break points in the conductometric titration curves. When background electrolyte is added, ψ decreases and the effects of released hydrogen ions become more marked. As an example, Fig. 6.17 shows the titration of a sulfite pulp in sodium chloride of various concentrations.

If the effects of the Donnan potential are taken care of in this way, conductometric titration becomes a very accurate method for determining the concentrations of strong and moderately weak acids in fibers. However,

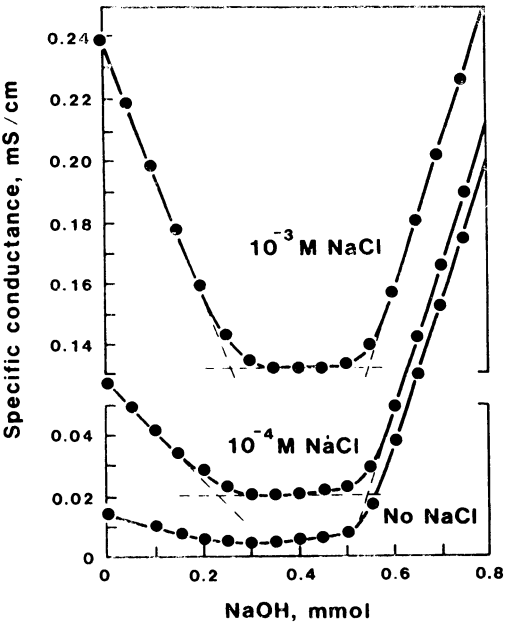

Fig. 6.17. Conductometric titration of sulfite pulp in water and in sodium chloride solution of different concentrations. The two intersection points of the extrapolated dashed lines (10^{-3} M NaCl) correspond to the contents of sulfonic (left) and total (right) acidic groups. (Scallan et al. 1988)

when the acid groups are very weak, the contributions of hydrogen ions to the total conductivity may become so small that accurate titrations are not possible.

6.6.3 Potentiometric Titration

As noted above, potentiometric titration in combination with ion exchange is used extensively for determination of the total amount of acid groups in fibers. However, as is the case for conductometric titration, potentiometric titration can also be used for the direct determination of the amounts of different acid groups in the fibers. Titrations of fibers in suspensions with low background electrolyte yield titration curves with slopes that change slowly over the whole pH range. Hence, it may be difficult to identify the inflection points corresponding to neutralization of acids with different dissociation constants. The reason is the dependence of the apparent dissociation constant on the degree

of dissociation, as discussed above. The electrostatic effects can be reduced by adding background electrolyte, in the same way as for conductometric titrations. More importantly, a full analysis of potentiometric titration curves can yield quite detailed information on the amounts and dissociation constants of strong, weak, and very weak acids in the fibers and also on the surface potential, i.e., on the Donnan equilibrium and changes in the apparent dissociation constant due to electrostatic effects. Such information is not directly accessible by conductometric titration or chemical analysis, but is essential if quantitative prediction of ion binding as a function of both pH and ionic strength is desired, as may be the case, e.g., in describing washing and dewatering processes.

In most cases, potentiometric titrations of fibers have been interpreted using conventional polyelectrolyte theory, assuming that the fibers contain only one (carboxylic) acid. In this case, the standard Henderson-Hasselbalch analysis of polyelectrolyte titrations can be used. From Eq. (6.12), the expression

$$pH = pK + log\frac{[B]}{[HB]} + \frac{F\psi}{RT\,In10} \tag{6.18}$$

can easily be derived.

Thus, the intrinsic dissociation constant K can be determined by extrapolation of a plot of log([B]/[HB]) versus pH to log([B]/[HB]) = 0. The plot is usually curved, due to the dependence of ψ on the degree of dissociation. It becomes more linear if a background electrolyte is used. However, if the fibers contain two or more acids with different dissociation constants, the simple Henderson-Hasselbalch approach is simply not valid. Methods to evaluate experimental titration curves in terms of multiple equilibria were devised many years ago (e.g., Sillén 1956) but, surprisingly, have not until very recently been applied to fibers. Essentially, one proceeds as follows.

If the total concentration of protons (free and bound to acids), C_H, the total concentration of acids, C_B, and the concentration of free hydrogen ions (pH) are known, an experimental value for the quantity Z in Eq. (6.13) may be calculated from

$$Z_{exp} = \frac{C_H - \{H\} + K_W/\{H\}}{C_H}, \tag{6.19}$$

where K_w is the ionic product of water. A constant background electrolyte concentration should be used so that variations in activity coefficients are minimized. Very accurate values of C_H and C_B can be determined by adding a known excess of hydrogen ions to a neutral pulp suspension followed by back-titration with alkali. The concentration of free and bound hydrogen ions should be determined using Gran plots (Gran 1950). Once the Z_{exp}

are known, they may be fitted to different models of the acid composition in the fiber described by Eq. (6.13), using the dissociation constants and the total charge on the fibers (which determines ψ) as adjustable parameters. For this purpose, several computer programs, e.g., FITEQL (Westall 1982) are available.

Figure 6.18 shows an example of a titration of an unbleached kraft pulp. It is found that the fibers contain two types of acids that dissociate in the pH interval 2.5–8: one with pK 3.4, and another with pK 5.5. For a large number of different fibers, the amount of the latter, rather weak acid, correlates directly with the amount of lignin in the fibers. However, its exact nature has not been clarified.

Thus, potentiometric titration is able to yield very precise information on the amounts and dissociation constants of different acids in the fibers, even if they contain mixtures of weak acids. It should be stressed that because neither conductometric nor potentiometric titration is able to yield any information on the structure of the acids, these methods should be supplemented by independent information on the chemical structure of the dissociating groups.

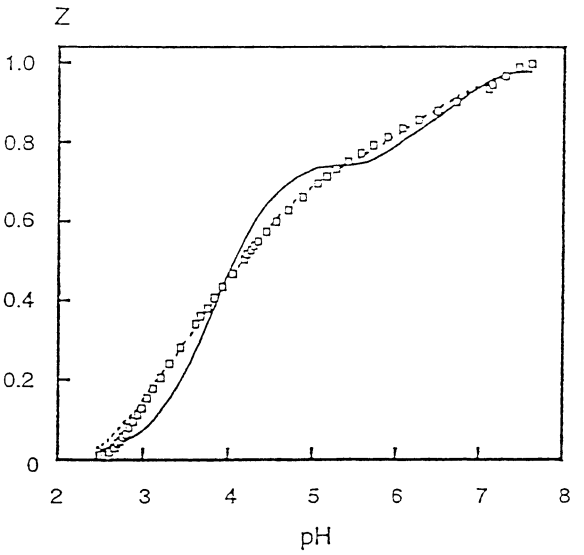

Fig. 6.18. Potentiometric titration of unbleached softwood kraft pulp (kappa number 19) suspended in 0.1 mol dm^{-3} NaCl. □: experimental data; ------ "best possible" theoretical fit of a model assuming that two acids occur in the fiber with pK values of 3.4 and 5.5, corrected for electrostatic interactions; — the same, without electrostatic correction. (Laine et al. 1994)

6.6.4 Polyelectrolyte Adsorption

It was shown by Wågberg et al. (1985) that adsorption of highly charged cationic polyelectrolytes can be used to determine the total charge on fibers. The method is based on the assumption that the polyelectrolyte charges react stoichiometrically with the charges in the fibers. This will be the case if the fibers and polymers are highly charged and the fibers are sufficiently swollen to allow the polyelectrolyte to diffuse into the fiber wall. Provided these conditions are fulfilled, the charge determined by polyelectrolyte adsorption correlates quite well with the amounts determined by potentiometric or conductometric titration.

The determination involves the following steps. Pulp that has been fully converted to sodium ion form is immersed in dilute solution, and pH is adjusted to the desired value. An appropriate amount of polyelectrolyte is added, and the suspension is equilibrated. After separation of the fibers, the remaining amount of polycation in solution is determined by titration with a standard anionic polyelectrolyte solution. The end point is detected with a dye indicator or instrumentally. A sufficient number of different initial polycation concentrations is used so that an adsorption isotherm can be plotted (Fig. 6.19). After a sharp initial rise, the isotherms generally level off to a slowly rising level. This slow rise is generally attributed to overcompensation of the charge as the equilibrium polymer configuration undergoes changes. A value for the stoichiometrically equivalent amount of polymer is obtained by extrapolation of the slowly rising level to zero concentration.

It should be stressed that the equilibrium configuration of adsorbed polyelectrolytes depends strongly on the charge densities of the surface and the polymer, on the ionic strength and on the molecular weight of the polymer. Care should therefore be taken to choose experimental conditions so that a stoichiometric correspondence between surface and polyelectrolyte charge is ensured.

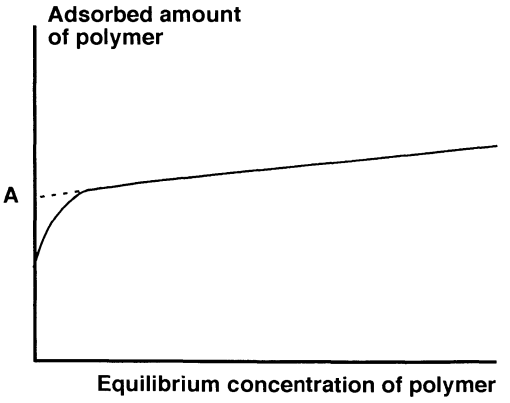

Fig. 6.19. Schematic adsorption isotherm for cationic polymer on fibers, showing the extrapolation to zero concentration (point A) used for evaluation of the stoichiometrically equivalent amount of polymer. Adapted from Wågberg et al. 1985

Polyelectrolyte adsorption is a rather cumbersome way to determine total charge compared with potentiometric or conductometric titration. One essential advantage of the method is that not only the amount of charge but also its accessibility can be determined. Thus, using polyelectrolytes of different molecular weights, different parts of the porous fibers will be accessible. Low-molecular weight polymers (molecular weight below, say, 10000) generally indicate fiber charges that are a few percent higher than those measured by titration. This indicates that all charges in the fiber are accessible to the polymer and that, indeed, some overcompensation may occur. When using polymers of higher molecular weight, lower charges are indicated. It appears that if molecular weights larger than about 2×10^5 are used, polyelectrolyte adsorption probes only the charge on the outer surface of the fibers. An example of the dependence of adsorption on molecular weight is given in Fig. 6.20.

6.7 Pyrolysis Gas Chromatography

At high temperatures, wood polymers are fragmented into small molecules through homolytic or heterolytic reactions. The reaction products often carry information on the structure of their parent polymers. Different techniques are available that combine the heat treatment and analytical separation and/or identification of the products. Among these, pyrolysis gas chromatography (Py-GC) is most widely applied.

In Py-GC the sample (<0.1 mg) is generally heated rapidly to a high temperature in an inert atmosphere. During a short period of pyrolysis (<10 s) an

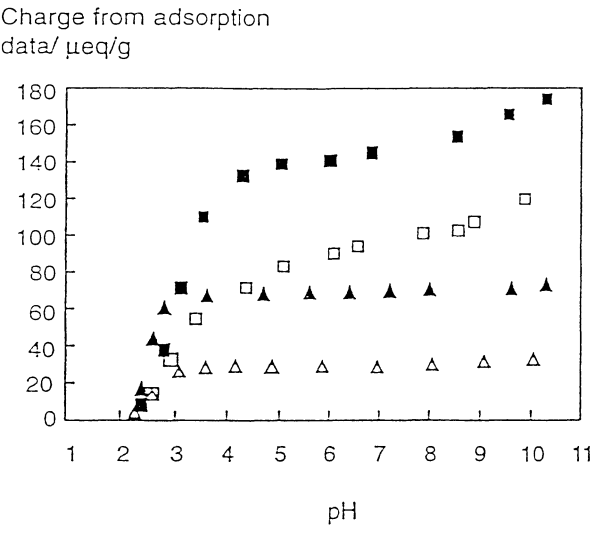

Fig. 6.20. Adsorption of cationic polyelectrolytes on unbleached birch (■, ▲) and pine (□, △) kraft pulps. ■, □: molecular weight of polymer 8×10^3; ▲, △: molecular weight 2×10^5. (Laine et al. 1996)

inert gas stream flushes the volatile reaction products into a GC column where they are condensed. The products are normally separated by using oven temperature programming and are typically identified by mass spectrometry (mass selective detection).

The major pyrolysis products of wood hexosans and pentosans are 1,6-anhydrohexopyranoses (1,6-anhydroglucopyranose, 1,6-anhydromannopyrase, and 1,6-anhydrogalactopyranose) and 1,4-anhydropentopyranoses (1,4-anhydroxylopyranose, and 1,4-anhydroarabinopyranose), respectively (Shafizadeh and Fu 1973; Helleur 1987; Fig. 6.21). It is noteworthy that 1,4-anhydroarabinopyranose is formed although arabinose exists mainly in the furanose form in wood polysaccharides. Partial change of ring size also occurs in pyrolysis of cellulose which yields minor amounts of 1,6-anhydroglucofuranose. Therefore, the formation of anhydrosugars must follow at least partly an S_N1 mechanism. Because S_N1 reactions are not stereospecific, information on anomeric carbon configuration is lost during pyrolysis. In summary, quantification of the anhydrosugars from wood and pulp samples can only be used for determination of their monosaccharide composition. More detailed structural determination would require analysis of oligomeric pyrolysis products, which are not volatile enough for separation on GC.

The carbonium ion intermediates (S_N1) in anhydrosugar formation can alternatively undergo retro-aldo reaction and dehydrogenation (Arisz et al. 1990). These reactions yield, after additional steps, glycolaldehyde, 1,5-anhydro-4-deoxypent-1-en-3-ulose and 1,5-anhydro-4-deoxyhex-1-en-3-ulose, respectively. Dehydrogenation is especially prominent with xylans because substitution at *O*-4 prevents early formation of 1,4-anhydroxylopyranose end groups. Because the formation of anhydrosugars is incomplete and varies in extent, depending on the structure of polysaccharide, determination of monosaccharide composition by Py-GC must be calibrated with other methods.

Pyrolysis reactions of lignin are not as well understood as those of polysaccharides. Bond cleavages occur mainly in the aliphatic side chains of phenylpropane units while oxygen substituents at the aromatic ring are retained. Based on the oxygen substitution pattern of monomeric fragmentation products of lignin, its origin can be classified according to the proportions of *p*-phydroxyphenyl, guaiacyl, and syringyl units (Meier and Faix 1992).

Attempts have also been made for a more detailed structural analysis of lignins, especially residual lignin in pulps, by Py-GC. A successful analysis of condensed lignin structures would require separation of less volatile oligomeric fragmentation products, which is not best suited to gas chromatography.

The pyrolysis yields of volatile products of both polysaccharides and lignin are relatively high, and hence their proportions in wood and pulp samples can reliably be determined by Py-GC. The optimum performance requires, however, good control of metal ion concentrations and temperature as both

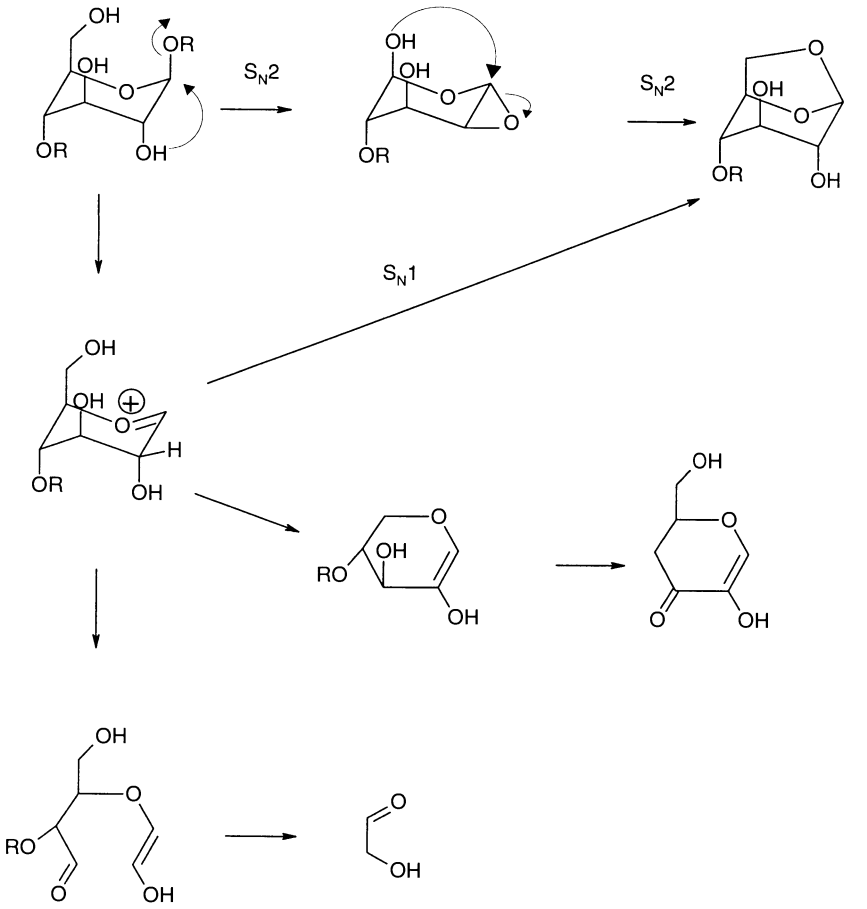

Fig. 6.21. Fragmentation of cellulose during pyrolysis. 1,6-Anhydroglucopyranose is formed through S_N2 and S_N1 reactions. The oxonium ion intermediate (S_N1) may also be degraded through a retro-aldo reaction or dehydrogenation followed by a β-alkoxy elimination yielding glycolaldehyde and 1,5-anhydro-4-deoxyhex-1-en-3-ulose, respectively

variables affect fragmentation rates through different pathways (Kleen 1993; Alén et al. 1996).

6.8 Guideline for Selection of Methods

Table 6.8 summarizes the different methods of analysis described in this chapter. Emphasis has been on the characterization of the chemical properties of fibers. Determinations of physical properties, such as crystallinity, porosity,

Table 6.8. An overview of methods for analysis of fiber composition

Method	NMR	FTIR	Raman	UV/Vis	Py-GC	ESCA	SIMS	Wet
Spatial resolution (μm)	–	10	1	0.1	–			–
Analytical depth (nm)	–	$10–10^5$			–	1–10	1	–
Reflectance measurement	–	Yes	Yes	Yes	–	Yes	Yes	–
Transmittance measurement	–	Yes	No	Yes	–	No	No	–
Gross chemical composition	Yes	Yes	Yes	No	Yes	No	No	No
Lignin content	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Detailed chemical structure	Yes	Yes	Yes	No	Yes	No	No	Yes
Physical state of cellulose	Yes	Yes	Yes	No	No	No	No	No
Orientation of wood polymers	Yes	Yes	Yes	No	No	No	No	No

cell wall structure, and surface energy, which are required for a comprehensive description of fiber structure and chemistry, have not been discussed. However, in those cases where some information about the physical properties can be obtained by the methods described above, these have been included in Table 6.8.

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7 Characterization of Pulping Liquors

K. Niemelä and R. Alén

7.1 Introduction

The spent liquors from chemical pulping contain varying amounts of organic compounds derived from all wood constituents (Table 7.1). The nature and amount of these compounds depend on the pulping method and on the raw material used for the pulp production.

During recent decades, these liquors have been analyzed for both high- and low-molecular weight compounds for numerous reasons, and depending on the scope of the investigations, only certain compounds or compound groups have usually been studied. Very seldom, if ever, has there been a specific need to analyze any liquor samples comprehensively for all organic compounds, as this would mean analysis of hundreds of different constituents. So far, investigations on spent liquors have been focused mainly on the following areas:

1. Basic understanding of pulping chemistry,
2. Physical properties,
3. On-line or off-line cooking control,
4. Isolation or production of useful by-products,
5. Environmental impacts.

During kraft (sulfate) pulping, or generally during the conditions prevailing at alkaline pulping, a substantial part of the hemicelluloses are converted to hydroxy carboxylic acids, although numerous other low-molecular weight compounds are also formed. Small amounts of dissolved polysaccharides are not completely degraded and can be found in the final black liquors. Analysis of the black liquors for polysaccharides and their degradation products is discussed in Section 7.2.1. During the acid sulfite pulping the carbohydrate losses are mainly due to acid-catalyzed hydrolysis of the polysaccharides to oligo- and monosaccharides, followed by their partial oxidation to aldonic acids. Some dehydration of the monosaccharides to carbonyl and other compounds also takes place. Identification of these compounds in sulfite spent liquors is discussed in Section 7.3.1.

Dissolution and degradation of lignin during both kraft and acid sulfite pulping produce a complex mixture of the breakdown products, with wide molecular weight distributions, ranging from simple phenolic compounds to large macromolecules. A large number of monomeric degradation products, sometimes known as monolignols, have been identified in both types of liquors, but the oligomeric products have received much less attention.

E. Sjöström et al. (eds.), *Analytical Methods in Wood Chemistry, Pulping, and Papermaking*

Table 7.1. The main classes of compounds derived from the wood constituents during kraft (or soda) and acid sulfite pulping

Wood constituent(s)	Kraft pulping		Sulfite pulping	
	Polymer fraction	Monomer fraction	Polymer fraction	Monomer fraction
Polysaccharides	Hemicelluloses (deacetylated)	Aliphatic carboxylic acids, cyclic compounds	Oligo- and polysaccharides	Monosaccharides, aldonic acids, other acids, carbonyl compounds
Lignin	Kraft lignin	Phenolic compounds	Lignosulfonates	Sulfonated and nonsulfonated phenols
Extractives	Polyphenols[a]	Fatty acids, terpenoids, steroids, other compounds	Polyphenols[a]	Fatty acids, terpenoids, steroids, lignans, other compounds
Proteins	Poorly known	Aliphatic and cyclic compounds	Poorly known	Poorly known
Suberin	Poorly known	Aliphatic acids and alcohols	Poorly known	Poorly known

[a] Mainly in certain tropical hardwoods.

Analysis of these compounds is discussed in Sections 7.2.2 and 7.3.2, together with a short discussion of the corresponding polymeric fractions, kraft lignin and lignosulfonates.

The extractives present in wood may enter the pulping liquors either in their original forms, or various hydrolysis or other transformation reactions may occur. The composition of the fraction derived from the extractives strongly depends on the wood material and on the pulping methods applied. For example, fatty acids are liberated from their esters during kraft pulping and enter the black liquor, but the esters remain mainly undissolved during sulfite pulping. Analysis of selected extractives in kraft and sulfite spent liquors will be discussed in Sections 7.2.3 and 7.3.3, respectively.

In addition to the above main compounds, the pulping liquors contain numerous minor low-molecular weight compounds derived from wood proteins or from suberin, or their origin is unclear. Determination of these relatively poorly known compounds in black liquors will be discussed in Section 7.2.4.

The isolation of polysaccharides, lignin, and extractives from pulping liquors, followed by their further characterization, usually follows the same procedures as described elsewhere in this book. The particular attention of this chapter is focused on the determination of low-molecular weight degradation products from carbohydrates and lignin. The methods used for the determination of inorganic components (Sect. 7.4) and the characterization of the physical properties (Sect. 7.5) of pulping liquors are finally described briefly. Analytical methods applied to the waters and effluents from papermaking, including components from mechanical and chemimechanical pulps, are described in Chapter 9.

7.2 Kraft Black Liquors

Examples of typical kraft black liquor compositions are given in Table 7.2. Although most of the black liquors have relatively similar compositions, at least for the main compound types, it should be remembered that only a few black liquors have so far been investigated in any detail. Thus, for example, very little is currently known about the composition of black liquors derived from alkaline pulping of tropical hardwoods or non-wood materials.

7.2.1 Polysaccharides and Their Degradation Products

Although the dissolved polysaccharides are mainly degraded to monomeric compounds, the black liquors usually contain some undegraded or partially depolymerized polysaccharides.

Table 7.2. Typical composition (%) of the dry matter of pine and birch kraft black liquors

Component	Pine	Birch
Lignin[a]	31	25
HMW (>500 Da) fraction	28	22
LMW (<500 Da) fraction	3	3
Aliphatic carboxylic acids	29	31
Formic acid	6	4
Acetic acid	4	8
Hydroxy monoacids	16	17
Hydroxy diacids	3	2
Other organics	7	11
Extractives	4	3
Polysaccharides	2	7
Miscellaneous	1	1
Inorganics	33	33
Sodium bound to organics	11	11
Inorganic compounds	22	22

[a] HMW and LMW refer to high-molecular weight and low-molecular weight, respectively.

7.2.1.1 Polysaccharides

Polysaccharide fractions have traditionally been isolated from neutralized kraft black liquors, after removal of lignin, by precipitation with ethanol (Simonson 1971). Determination of the carbohydrate composition shows that the dominating polysaccharide is usually xylan, although the separation of individual hemicelluloses in pure form from these preparations has seldom been attempted. Some incorporated lignin is usually also present in these preparations.

More detailed analysis of black liquor hemicelluloses (xylan, arabinan, and galactan) can be achieved by precipitating them with 1,4-dioxane, followed by chromatographic fractionation on anion exchange resins (Engström et al. 1995).

7.2.1.2 Types of Degradation Products

Various carboxylic acids which form the main carbohydrate-derived degradation products are listed in Table 7.3, but numerous other types of compounds are also formed (Table 7.4).

Table 7.3. The main classes of carbohydrate-derived carboxylic acids in alkaline pulping liquors, and number of identified compounds

Compound type	Number
Volatile fatty acids	4
Hydroxy monocarboxylic acids	60
Dimeric hydroxy monocarboxylic acids	11
Dicarboxylic acids	10
Hydroxy dicarboxylic acids	30
Hydroxy tricarboxylic acids	3

Table 7.4. Miscellaneous carbohydrate-derived compounds in alkaline pulping liquors, and number of identified compounds

Compound type	Number
Catechols	5
Cyclopentenones	7
Hydroxycyclopentenones	12
Thiophenes	20
Miscellaneous compounds	10

7.2.1.3 Volatile Fatty Acids

The main volatile fatty acids include acetic and formic acids. Varying methods have been used for their determination – the most recent ones include gas chromatography (GC) and ion chromatography (IC). The determination of these acids by GC usually requires a successful work-up procedure and an esterification method, although direct aqueous injection may also be applied. Due to the high volatility of acetic and formic acids, significant losses during sample preparation may easily take place. In one of the most successful methods, losses have been prevented by preparation of benzyl esters via *tert*-butyl ammonium salts (Bethge and Lindström 1974; Alén et al. 1985a). This procedure has frequently been used to analyze volatile fatty acids in alkaline pulping liquors. In an alternative potential method, nonvolatile triethylamine adducts may be prepared from acetic and formic acids, which are subsequently *tert*-butyldimethylsilylated (Kim et al. 1987). This method has not yet, however, been applied to black liquor. As a whole, a huge number of GC methods have been described for the determination of acetic and formic acids in aqueous samples, of which many could apparently be adopted for black liquor investigations.

IC provides a promising alternative for the determination of acetic and formic acids in black liquors, although it is mainly applied to the analysis of

inorganic anions (Franklin 1982; Krishnagopalan et al. 1985). The main benefits include ease of operation, speed of analysis, and lack of any derivatization steps.

In addition to acetic and formic acids, minor amounts of other volatile fatty acids (C_3–C_6 acids) are apparently present in all alkaline pulping liquors (Kosyukova and Galyanova 1983). Their origins are not fully clear, but at least some propanoic and butanoic acids may be derived from carbohydrates. As their determination has so far received only scattered attention, no procedures have yet been developed and optimized. Certain wood species are known to contain relatively large amounts of some of these fatty acids (Sandermann et al. 1970), and thus unusually large amounts of them may be encountered in the corresponding spent liquors.

7.2.1.4 Hydroxy Carboxylic Acids

The main nonvolatile carboxylic acids include numerous hydroxy monocarboxylic and hydroxy dicarboxylic acids, but minor amounts of non-hydroxylated di- and tricarboxylic acids have also been identified.

During the past 15–20 years, the complex nature of the hydroxy acid fraction in alkaline pulping liquors has become firmly substantiated, as altogether nearly 100 such compounds have been identified (Niemelä and Sjöström 1986c; Niemelä 1990b, 1993b). Examples of the occurrence of these polar acids in black liquors are given in Table 7.5. Currently, the only method that allows a successful separation of these compounds (or most of them) is high resolution capillary GC, or preferentially, GC/MS.

Sample Preparation for GC. Simple procedures are available for the isolation and conversion of the hydroxy acids to a form amenable to GC. In a useful method (Alén et al. 1984), a sample of black liquor is passed through a cation exchange (NH_4^+) column. The resulting aqueous solution of ammonium carboxylates is evaporated to dryness, per(trimethylsilyl)ated and analyzed by GC or GC/MS. The main benefit of the use of ammonium salts is that complete or partial lactonization of certain hydroxy acids can be avoided, although there may be certain cases when it is desirable to analyze the hydroxy acids in the form of lactones.

Trimethylsilylation of the ammonium carboxylates readily occurs at room temperature in pyridine with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), containing a few percent of trimethylchlorosilane (TMCS). A good number of alternative trimethylsilylation reagents and solvents are also available (see Table 3.2 on page 52) (Evershed 1993).

Using the procedure described above, only small black liquor volumes are usually required, typically 0.1–0.5 ml. Although this is the recommended general procedure (for the applications, see Alén et al. 1985b, 1991; Niemelä et al. 1985; Niemelä and Sjöström 1986c; Alén 1988a,b; Niemelä 1988a, 1989d,

Table 7.5. Examples of the main nonvolatile polar carboxylic acids in pine (*Pinus sylvestris*), birch (*Betula verrucosa/pubescens*, and eucalypt (*Eucalyptus grandis*) kraft black liquors (Alén et al. 1985b; Niemelä et al. 1985; Niemelä 1991). All values are in g/l

Carboxylic acid	Pine	Birch	Eucalypt
Monocarboxylic acids			
Glycolic	2.54	2.31	1.99
Lactic	4.20	3.83	2.65
3-Hydroxypropanoic	0.02	0.19	0.04
Glyceric	0.13	0.11	0.08
2-*C*-Methylglyceric	0.10	0.12	0.13
2-Hydroxybutanoic	1.04	6.82	2.95
4-Hydroxybutanoic	0.19	0.10	0.08
2-Deoxytetronic	0.09	0.10	0.05
3-Deoxytetronic	0.26	0.59	0.36
2-Hydroxy-2-methylbutanoic	+[d]	+[d]	0.40
2-Hydroxypentenoic	0.30	0.15	0.16
3,4-Dideoxypentonic	2.25	1.18	1.21
3-Deoxypentonic[a]	1.46	0.88	0.81
Xyloisosaccharinic	0.48	3.76	1.90
Anhydroisosaccharinic[a]	0.34	0.18	0.13
3,6-Dideoxyhexonic[b]	0.16	0.56	0.29
3-Deoxyhexonic[c]	0.30	0.30	0.18
Glucoisosaccharinic[a]	8.97	4.11	3.48
Dicarboxylic acids			
Oxalic	0.13	0.17	0.42
Succinic	0.22	0.22	0.33
Methylsuccinic	0.18	0.04	0.16
Malic	0.16	0.27	0.19
2-Hydroxyglutaric	0.39	0.50	0.66
3-Deoxypentaric[a]	0.05	0.07	0.02
2-Hydroxyadipic	0.43	0.24	0.12
2,5-Dihydroxyadipic[a]	0.42	0.22	0.28
Glucoisosaccharinaric[a]	0.47	0.69	0.59

[a] *erythro* and *threo* isomers.
[b] *ribo* and *arabino* isomers.
[c] *ribo*, *arabino*, *xylo*, and *lyxo* isomers.
[d] + indicates concentrations below 0.02 g/l.

1990b, 1991), there is occasionally a need to prepare more concentrated samples for the detection of numerous minor compounds. Since the high-molecular weight materials (lignin and polysaccharides) are also present in the sample, more concentrated samples cannot always be prepared simply by increasing the black liquor volume and keeping the volume of the derivatization mixture constant. Instead, higher liquor volumes (up to several ml) should be freed from lignin and polysaccharides by mixing diluted samples with a strong cation exchange resin (in H^+-form), followed by filtration. Subsequent

addition of some ammonia solution to the filtrate prior to evaporation ensures that ammonium carboxylates are obtained for trimethylsilylation. It is important to add the ammonia before the evaporation. If the evaporation is carried out first, the subsequent conversion of lactone-forming acids to ammonium salts, by ammonia treatment, will suffer from competing formation of amides.

On the other hand, if only the main hydroxy carboxylic acids need to be analyzed, there is an even simpler procedure that can be applied, since it is also possible to trimethylsilylate aqueous solutions of sodium carboxylates. Accordingly, a small black liquor volume (20–50 μl) is readily derivatized by injecting it in to a trimethylsilylation mixture. After a short reaction time, the mixture is ready for GC or GC/MS analyses.

The use of *tert*-butyldimethylsilylation has recently become more and more popular in carboxylic acid analysis. The main advantage of these derivatives is that they are significantly more resistant toward hydrolysis than the TMS derivatives are. However, their suitability for black liquor investigations is questionable because of difficulty of derivatization of polyhydroxy compounds, significantly increased molecular weights (and thus retention times) of the derivatives, and less comprehensively known mass spectrometry of the derivatives.

As already mentioned, the hydroxy acids can also be derivatized without converting them into their ammonium salts (Malinen and Sjöström 1975), which means that the acids are trimethylsilylated either as the free acids or as the lactones. This alternative can provide a number of benefits and is valuable as a supplementary source of information. In this procedure, a black liquor sample of a suitable volume (typically 0.1–0.5 ml) is first passed through a cation exchange (H^+-form) column and evaporated to dryness, and the remaining acids are derivatized. A complex chromatogram will inevitably be obtained, as many hydroxy acids are only partially lactonized. However, many of these compounds will become completely lactonized if some hydrochloric acid is added to the filtrate prior to the evaporation. For example, all 3-deoxyaldonic and isosaccharinic acids are completely or nearly completely lactonized, and, importantly, many of them are much better resolved as lactones than as their open-chain counterparts. Many identifications can also be confirmed if the same compounds can be detected and identified in different forms. The main disadvantage of this procedure is, however, that some compounds, such as 4-hydroxybutanoic and 4-hydroxypentanoic acids, will remain totally undetected due to the volatility of their lactones. Also, many compounds, such as 2-hydroxyglutaric and 2,5-dihydroxyadipic acids, are only partly lactonized, thus producing several GC peaks.

A detailed GC/MS analysis of the lactonized samples often shows the presence of small amounts of monosaccharides, such as xylose, arabinose, and glucose. They are liberated from indigenous oligo- or polysaccharides during acidification and evaporation, and do not therefore represent genuine black liquor compounds.

Gas Chromatography. The derivatized mixtures can be analyzed using various chromatographic conditions and non-polar capillary columns, and no "best" conditions can be given. The initial oven temperature typically varies from 80 to 130 °C and the program rate from 5 to 15 °C/min. The final temperature and time should be adjusted, depending on the depth of the desired analysis. Several published reference chromatograms can be used as guideline examples (e.g., Alén et al. 1984; Niemelä and Sjöström 1986c; Niemelä 1988a). These examples have shown that the separation of more than 150 compounds in 20 min is possible. So far, no chromatograms have yet been published showing capillary GC separation of lactonized black liquor compounds. The chromatographic conditions should be chosen so that the first three hydroxy acids (i.e., lactic, 2-hydroxy-2-methylpropanoic, and glycolic acids) are sufficiently well resolved. These hydroxy acids can be expected to be present in all alkaline pulping liquors.

Relative retention times and retention indices have been listed for a large number of black liquor carboxylic acids on three liquid phases of different polarities (Petersson 1977). These data can be used for the final identification of several isomeric pairs, such as *erythro-threo* isomers, for which the mass spectra are identical.

Quantitative determinations can be based on any methods used with modern capillary GC and GC/MS techniques, but the addition of an internal standard(s) at an early stage is usually necessary. There are numerous suitable internal standards, for example, commercially available aldonic acids and alditols.

Mass Spectrometry. Electron impact ionization methods (at 70 eV) have been used almost exclusively in combined GC and MS investigations. Mass spectra of the trimethylsilyl derivatives of numerous black liquor carboxylic acids are available in the literature (as listed by Niemelä and Sjöström 1986c; Niemelä 1990b), but no comprehensive spectra collections have yet been compiled.

The published mass spectra are naturally useful for those compounds that are not readily available. Additional reference acid mixtures can be obtained by alkaline treatment of selected carbohydrates, such as rhamnose, glucose, cellobiose, xylan, and pectic acid. These samples cover nearly all black liquor hydroxy acids, and they can be used to optimize chromatographic conditions and to facilitate identification. Several reference chromatograms have been published in the literature (Niemelä and Sjöström 1985a, 1986a,b; Niemelä 1990a).

The nature of the mass spectra of the trimethylsilyl derivatives of carboxylic acids is not discussed here in detail, but they are demonstrated by two spectra shown in Fig. 7.1.

Alternative Methods. Although the methods described above, based on capillary GC/MS, have provided detailed information about carboxylic acids in black liquors, the past and potential value of other methods may be note-

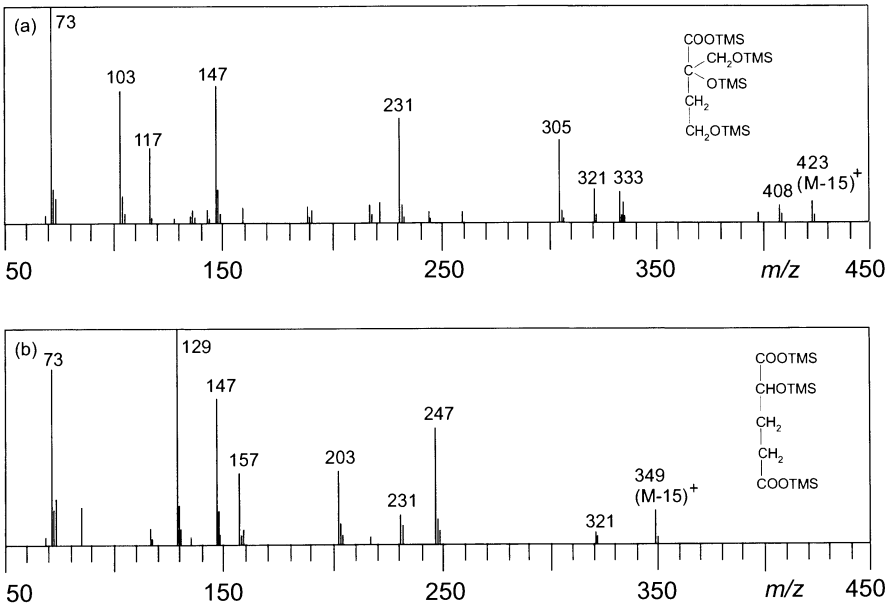

Fig. 7.1. The mass spectra at 70 eV of the trimethylsilyl derivatives of xyloisosaccharinic (**a**) and 2-hydroxyglutaric (**b**) acids

worthy. Particularly in the 1970s, a combination of high-performance liquid chromatography (HPLC) on ion exchange resins, followed by GC/MS on packed columns, provided much invaluable information (Löwendahl et al. 1976; Samuelson and Sjöberg 1978). No recent progress in this area has been reported, although modern liquid chromatography techniques would apparently make some excellent separations easily available.

IC has frequently been used for black liquor investigations. The main interest has been in inorganic anions, but the method has successfully been applied to detect some simple carboxylates, such as oxalate and lactate. If only a limited number of carboxylic acids are to be monitored, IC or capillary electrophoresis (CE) may prove to be versatile and rapid techniques.

Future Requirements. So far, only relatively few types of black liquors have been analyzed in any detail for the aliphatic hydroxy carboxylic acids (Table 7.6). A novel innovation resulting from these analyses is to use them for the control of delignification (Alén et al. 1991).

There is currently very little information about the hydroxy acids (or composition of organic compounds in general) in black liquors derived from non-wood material, such as bamboo, straw, or reed. Moreover, many important black liquors derived from hardwood and softwood and those from mixed species have so far received little attention. Although we can expect that such

Table 7.6. Alkaline pulping liquors, analyzed in detail for nonvolatile carboxylic acids

Raw material	Pulping method	Reference
Pinus sylvestris	Kraft	Löwendahl et al. (1976)
P. sylvestris	Soda-AQ	Samuelson and Sjöberg (1978)
P. sylvestris	Kraft and soda-AQ	Alén et al. (1985b)
P. sylvestris	Kraft	Niemelä (1988a)
P. sylvestris	Soda-alcohol	Alén (1988b)
Picea abies	Kraft and soda-AQ	Alén et al. (1985b)
Betula verrucosa	Oxygen-bicarbonate	Samuelson and Sjöberg (1976)
B. verrucosa/pubescens	Kraft and kraft-AQ	Niemelä et al. (1985)
B. verrucosa/pubescens	Soda-alcohol	Alén (1988a)
B. verrucosa/pubescens	Kraft	Niemelä (1990b)
Eucalyptus grandis	Kraft	Niemelä (1991)

liquors would contain mainly the same compounds as those found in the other liquors, several new findings are possible. For example, a eucalypt kraft black liquor was recently found to contain some novel aliphatic tricarboxylic acids (Niemelä 1991), apparently characteristic of this material. It is equally possible that other new compounds will later be discovered in black liquors not yet investigated. Nevertheless, the main hydroxy acids can be expected to be the same in all wood-derived black liquors.

Some dimeric hydroxy acids were recently discovered in certain black liquors, including several galactopyranosylsaccharinic acids (Niemelä 1989c). The determination of these types of acids requires higher oven temperatures (up to 260–280 °C) and wider MS scanning ranges (up to 900 u), but otherwise the same procedures can be applied as discussed above. The compounds identified are shown in Fig. 7.2. A large number of similar or related compounds, derived from arabinogalactan, are present in larch kraft black liquors and are still awaiting final identification.

7.2.1.5 Other Aliphatic Carboxylic Acids

The analysis of black liquors for polar, carbohydrate-derived carboxylic acids always results in the detection of minor amounts of other carboxylic acids, derived from sources such as fatty acids and lignin. These compounds, which include medium chain-length dicarboxylic acids and simple alkanetricarboxylic acids, occur in larger amounts in oxygen delignification liquors, indicating the important role of oxidative reactions for their formation. The tricarboxylic acids, of which 1,1,2-ethanetricarboxylic acid predominates, are found in the polar hydroxy acid fractions (Niemelä 1991). The dicarboxylic acids, in turn, may be isolated by solvent extraction and occur in the same

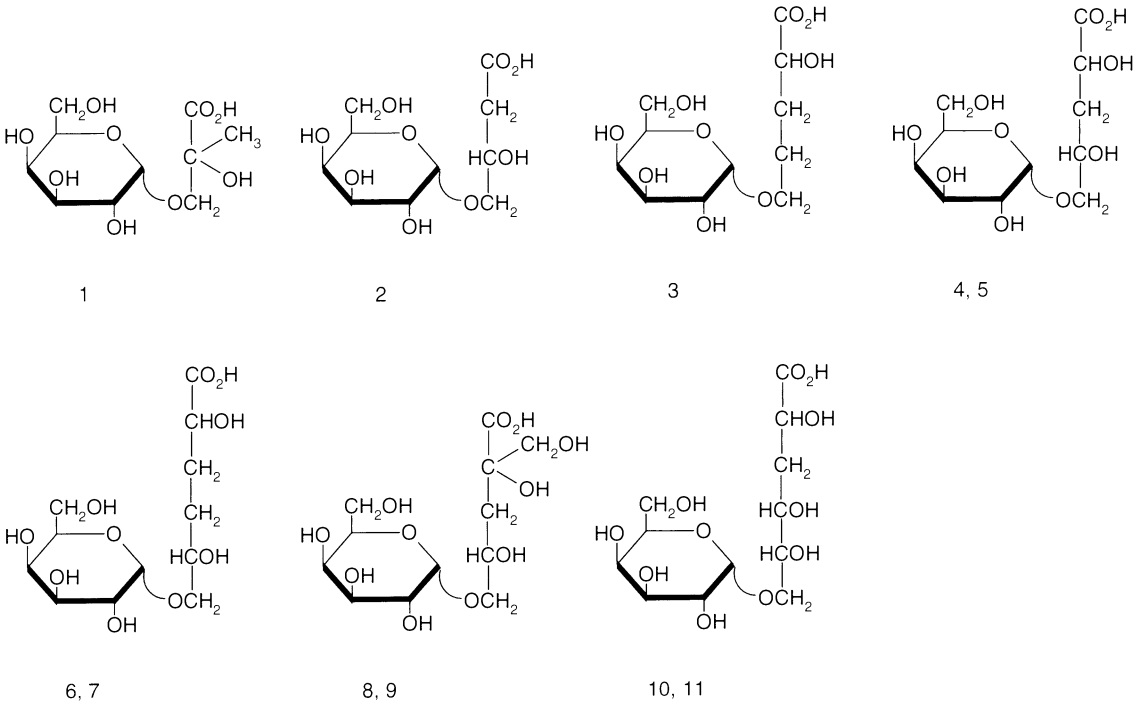

Fig. 7.2. Galactopyranosylsaccharinic acids identified in pine kraft black liquor. **1** 3-*O*-α-D-galactopyranosyl-2-*C*-methylglyceric; **2** 2-deoxy-4-*O*-α-D-galactopyranosyltetronic; **3** 3,4-dideoxy-5-*O*-α-D-galactopyranosylpentonic; **4** and **5** 3-deoxy-5-*O*-α-D-galactopyranosylpentonic (*erythro* and *threo* isomers); **6** and **7** 3,4-dideoxy-6-*O*-α-D-galactopyranosylhexonic (*erythro* and *threo* isomers); **8** and **9** 3-deoxy-5-*O*-α-D-galactopyranosyl-2-*C*-(hydroxymethyl)pentonic (*erythro* and *threo* isomers); and **10** and **11** 3-deoxy-6-*O*-α-D-galactopyranosylhexonic (*ribo* and *arabino* isomers) acids

fractions as longer-chain dicarboxylic acids derived from suberin (see Chap. 7.4).

7.2.1.6 Other Degradation Products

As already mentioned, degradation of polysaccharides also produces a wide variety of compounds of other types than carboxylic acids (Table 7.4). Most of them can readily be isolated from the black liquors by solvent extraction, for example with diethyl ether or chloroform. Simple thiophenes and cyclopentenones can be extracted from the original alkaline liquors, but acidification is required prior to the isolation of thiophenecarboxylic acids, hydroxycyclopentenones, and catechols (Niemelä 1988c, 1989a,b, 1990b; Berthold and Gellerstedt 1993). Further analysis is carried out by GC and GC/MS, and usually the fractions should be analyzed both with and without derivatization. One of the most suitable derivatization reactions is trimethylsilylation. Figure 7.3 shows examples of miscellaneous cyclic, carbohydrate-derived compounds typically found in black liquors.

7.2.2 Lignin and Its Degradation Products

7.2.2.1 Kraft Lignin

Lignins dissolved during alkaline pulping (alkali lignins) are usually known as kraft lignin or thiolignin (kraft pulping) and soda lignin (soda pulping). Their

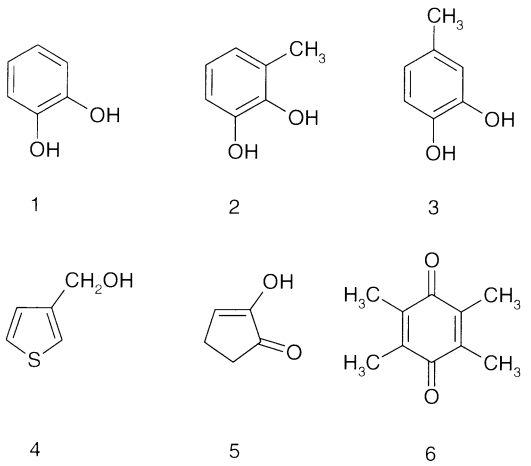

Fig. 7.3. Examples of carbohydrate-derived cyclic black liquor constituents. **1** catechol; **2** 3-methylcatechol; **3** 4-methylcatechol; **4** 3-thiophenemethanol; **5** 2-hydroxy-2-cyclopenten-1-one; and **6** 2,3,5,6-tetramethyl-1,4-benzoquinone

quantitative determination in the corresponding black liquors is done almost exclusively by UV spectrophotometry (Norrström 1970). Although these measurements may also provide information about certain functional groups in alkali lignins, more detailed characterization is usually done by other means after isolation of the lignin by precipitation. These methods currently include especially ^{13}C NMR and ^{1}H NMR spectroscopy for functional groups and structure elucidations, gel permeation chromatography (GPC) for molecular weight distribution, selected methods for phenolic hydroxyl groups, carbonyl groups and carboxyl groups, and oxidative degradations for interunit linkages. These methods are discussed in detail in Chapter 5.

The structural heterogeneity of alkali lignins is revealed only if careful fractionations are performed prior to the analytical characterizations. Usually, alkali lignins are fractionated after isolation and purification, for example by GPC or by successive solvent extractions (Mörck et al. 1986, 1988). It is also possible to isolate different fractions directly from black liquors, particularly by stepwise acidification or by ultrafiltration (Wada et al. 1962; Villar et al. 1996; Lappan et al. 1997).

Several tropical hardwoods are known to contain large amounts of polyphenolic extractives, which in pulping liquors affect the determination of lignin (Oye et al. 1977). No methods have yet been established to fully distinguish between these two aromatic fractions in the black liquors derived from such wood species.

7.2.2.2 Degradation Products of Lignin

Modern liquid and GC investigations have shown that black liquors contain hundreds of aromatic low-molecular weight compounds (Löwendahl et al. 1978; Niemelä 1990b). Although a large number of such compounds have been identified, the structure determination of many other compounds still challenges analytical chemists.

The low-molecular weight lignin-derived fragments form an extremely complex fraction of compounds with different basic structures and functional groups (Gierer and Lindeberg 1980; Gierer and Wännström 1984; Alén and Vikkula 1989a,b; Niemelä 1990b; Tai et al. 1990). Most of them are monolignols, but several dimers are also known. The monomer fraction is composed of several types of substituted phenols. The softwood black liquors contain almost exclusively guaiacyl compounds, and altogether more than 40 such compounds have already been identified (Table 7.7). The hardwood black liquors contain approximately the corresponding guaiacyl and syringyl compounds, but so far, only a relatively small number (approximately 10) of related 4-hydroxyphenyl compounds have been identified in such black liquors.

In addition to the above main aromatic compounds, black liquors contain minor amounts of some other phenolic compounds, such as catechols,

Table 7.7. Main classes and numbers of monomeric guaiacyl (4-hydroxy-3-methoxyphenyl) compounds identified in black liquors, and suggested derivatives for their GC determination

Guaiacyl compound type	Identified	GC derivatives
Alkyl derivatives	8	None, acetyl, TMS
Alkanol derivatives	10	Acetyl, TMS
Carbonyl compounds	7	None, acetyl, TMS
Carboxylic acids	6	TMS
Hydroxy carboxylic acids	7	TMS
Sulfur-bearing compounds	5	None, acetyl, TMS

methoxycatechols, or pyrogallol derivatives. Small amounts of nonphenolic compounds, such as benzoic acid, have been frequently reported.

Alkaline degradation of lignin also produces a variety of aliphatic low-molecular weight compounds, mainly carboxylic acids. Although the generation of these compounds is enhanced during oxygen-alkali delignification, they are also formed during the cooking stage. These products include various mono-, di-, and tricarboxylic acids, such as acetic, lactic, oxalic, malonic, succinic, ethanetricarboxylic, and tricarballylic acids. As many of them are better known as carbohydrate-derived products, their analysis has already been discussed in Section 7.2.1.

Isolation of the Aromatic Compounds. After adjusting the pH of the black liquor either to a neutral or to an acidic level, the aromatic compounds can be extracted using a suitable solvent, such as diethyl ether, ethyl acetate, or chloroform. The extracts can be investigated by various chromatographic and spectroscopic methods, or pure compounds can be isolated for more detailed studies. The most comprehensive separations have been achieved by GC or GC/MS.

Once a fraction of interest has been isolated, there are several suitable derivatization methods available prior to GC and GC/MS analyses, and the choice is normally based on the investigators' own preferences. The most frequently used methods include trimethylsilylation, acetylation or acetylation-methylation (Gierer and Wännström 1984), and ethylation. More comprehensive results are often obtained if most of the extracts are analyzed both with and without a derivatization. The main benefit of trimethylsilylation is that both of the carboxyl and hydroxyl groups are simultaneously derivatized. If the derivatization procedure does not allow esterification, carboxylic acids will remain undetected. On the other hand, esterification as the only method will leave most of the hydroxy and polyhydroxy compounds undetected. Thus, the choice of the derivatization method is crucial for a successful GC/MS investigation.

As summarized in Table 7.7, the simplest phenolic compounds include alkylphenols (such as guaiacol, syringol, and their 4-alkyl derivatives) and ketonic compounds (such as vanillin, acetovanillone, guaiacyl acetone, and the corresponding syringyl compounds). These can easily be recovered using many organic solvents, of which toluene is recommended, particularly if the recovery of more polar compounds is not desired. As these compounds contain only one hydroxyl group, they can readily be analyzed by GC without any preceding derivatization. If trimethylsilylation is used, there will be a slow but inevitable formation of trimethylsilylated enol ethers from oxo compounds, resulting in some extra GC peaks (Niemelä 1990b). Although this is by no means detrimental, it may cause some severe identification problems.

The main phenolic acids in black liquors include 4-hydroxybenzoic, vanillic, and syringic acids, although many others have also been found, such as guaiacyl- and syringylglyoxylic acids. These compounds can be isolated using many alternative solvents and are most conveniently analyzed by GC as their per(trimethylsilyl)ated (TMS) derivatives.

Numerous phenolic alkanols are present in black liquors, including 1-arylethanols, 2-arylethanols, 3-arylpropanols, 3-aryl-1,2-propanediols, and 1-arylglycerols. The compounds with only two hydroxyl groups can readily be recovered by solvent extraction. The more polar compounds, such as arylglycerols, remain in aqueous solution and can be analyzed, for example, in the unfractionated samples together with the aliphatic hydroxy acids.

Among the most polar aromatic black liquor compounds are phenolic hydroxy acids, and α-hydroxyarylalkanoic acids, all of which belong to recently discovered black liquor compounds (Löwendahl et al. 1978; Gierer and Wännström 1984; Niemelä 1988a,b, 1990b). Figure 7.4 shows the structures of such compounds found in birch kraft black liquor. Most of these acids are found in the fractions of aliphatic hydroxy acids (Sect. 7.2.1).

The nature of organically bound sulfur in kraft lignins is not yet fully understood (Nelson et al. 1963). This question can be approached by investigating sulfur-bearing lignin monomers and dimers in kraft black liquors. In a recent study (Niemelä 1990b), several methylthio groups containing guaiacyl and syringyl compounds were found in a birch kraft black liquor, suggesting that much of the sulfur may be bound in the form of methylthio groups.

All alkaline pulping liquors probably contain some lignin-derived catechol compounds, typically including catechol, 3,4-dihydroxybenzaldehyde, and 3,4-dihydroxybenzoic acid. Some minor catechols, such 3- and 4-methylcatechols, may also be derived from carbohydrates. Most of the catechol compounds can be isolated by solvent extraction for GC and GC/MS investigations. Many hardwoods, such as eucalypts, contain substantial amounts polyphenolic extractives, which produce decomposition products identical with or similar to those derived from lignin. No detailed studies have yet been conducted on such low-molecular weight compounds, but, for example, several catechols (Fig. 7.5)

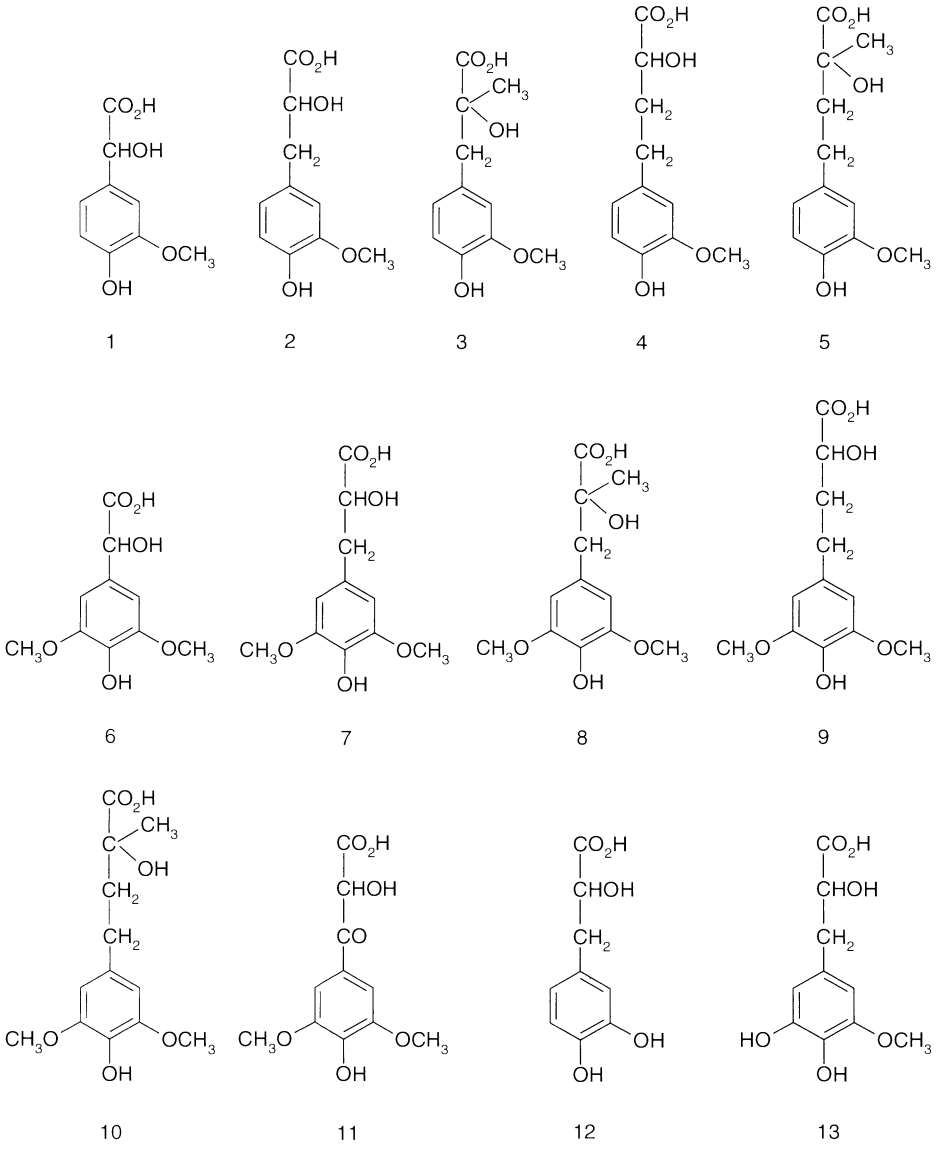

Fig. 7.4. Structures of α-hydroxyarylalkanoic acids identified in birch kraft black liquor. **1** guaiacylglycolic; **2** 3-guaiacyl-2-hydroxypropanoic; **3** 3-guaiacyl-2-hydroxy-2-methylpropanoic; **4** 4-guaiacyl-2-hydroxybutanoic; **5** 4-guaiacyl-2-hydroxy-2-methylbutanoic; **6** syringylglycolic; **7** 2-hydroxy-3-syringylpropanoic; **8** 2-hydroxy-2-methyl-3-syringylpropanoic; **9** 2-hydroxy-4-syringylbutanoic; **10** 2-hydroxy-2-methyl-4-syringylbutanoic; **11** 2-hydroxy-3-oxo-3-syringylpropanoic; **12** 2-hydroxy-3-(3,4-dihydroxyphenyl)propanoic; and **13** 2-hydroxy-3-(3,4-dihydroxy-5-methoxyphenyl)propanoic acids

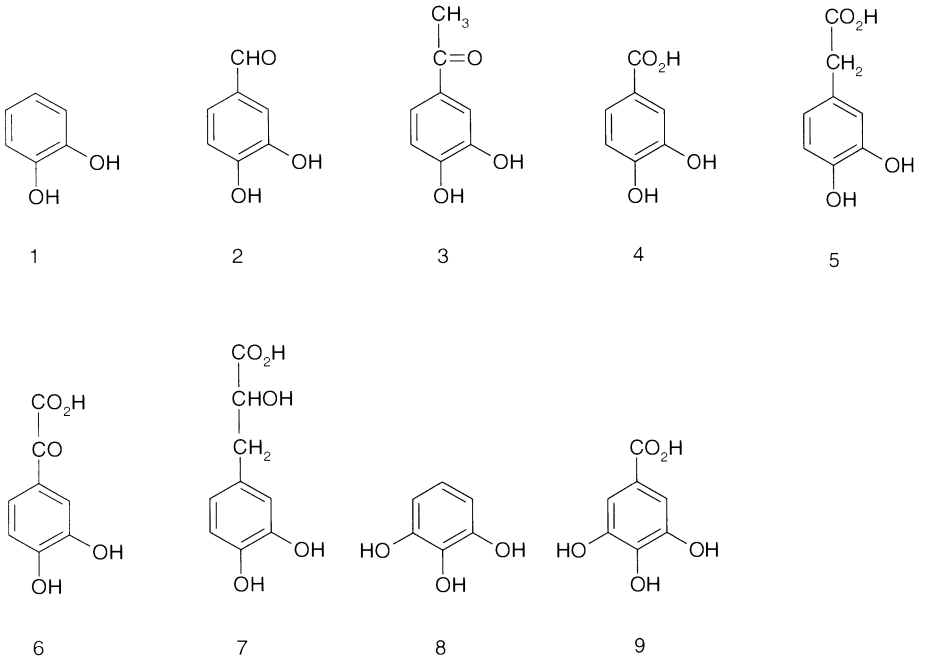

Fig. 7.5. Structures of catechol compounds found after alkaline digestion of jarrah (*Eucalyptus marginata*). Most of these compounds are probably derived from tannin-related extractives. **1** catechol; **2** 3,4-dihydroxybenzaldehyde; **3** 3,4-dihydroxyacetophenone; **4** 3,4-dihydroxybenzoic acid; **5** 3,4-dihydroxybenzeneacetic acid; **6** 3,4-dihydroxyphenylglyoxylic acid; **7** 2-hydroxy-3-(3,4-dihydroxyphenyl)propanoic acid; **8** pyrogallol; and **9** gallic acid

have been found in relatively large amounts after alkaline digestion of *Eucalyptus marginata* (K. Niemelä, unpubl.) and are obviously derived from the polyphenolic extractives. These compounds were isolated by ethyl acetate extraction and were analyzed by GC/MS as their per(trimethylsilyl) derivatives.

Detailed HPLC, GC, and GC/MS investigations of softwood and hardwood black liquors have revealed the presence of at least 200–300 dimeric aromatic compounds. Their identification would obviously provide valuable information on the reactions of lignin during pulping, and also on the distribution of structural units in alkali lignins. However, there are currently only limited possibilities for identifying all of them without time-consuming isolation, purification, and careful structure determinations. Examples of identified dimeric lignin-derived compounds are given in Fig. 7.6 (Gierer and Lindeberg 1980; van der Klashorst and Strauss 1986, 1987a,b; Niemelä 1988b, 1989d, 1990b; Berthold and Gellerstedt 1993).

Lignans form an additional source of phenolic compounds in certain softwood species, especially in spruce (*Picea*), larch (*Larix*), and hemlock

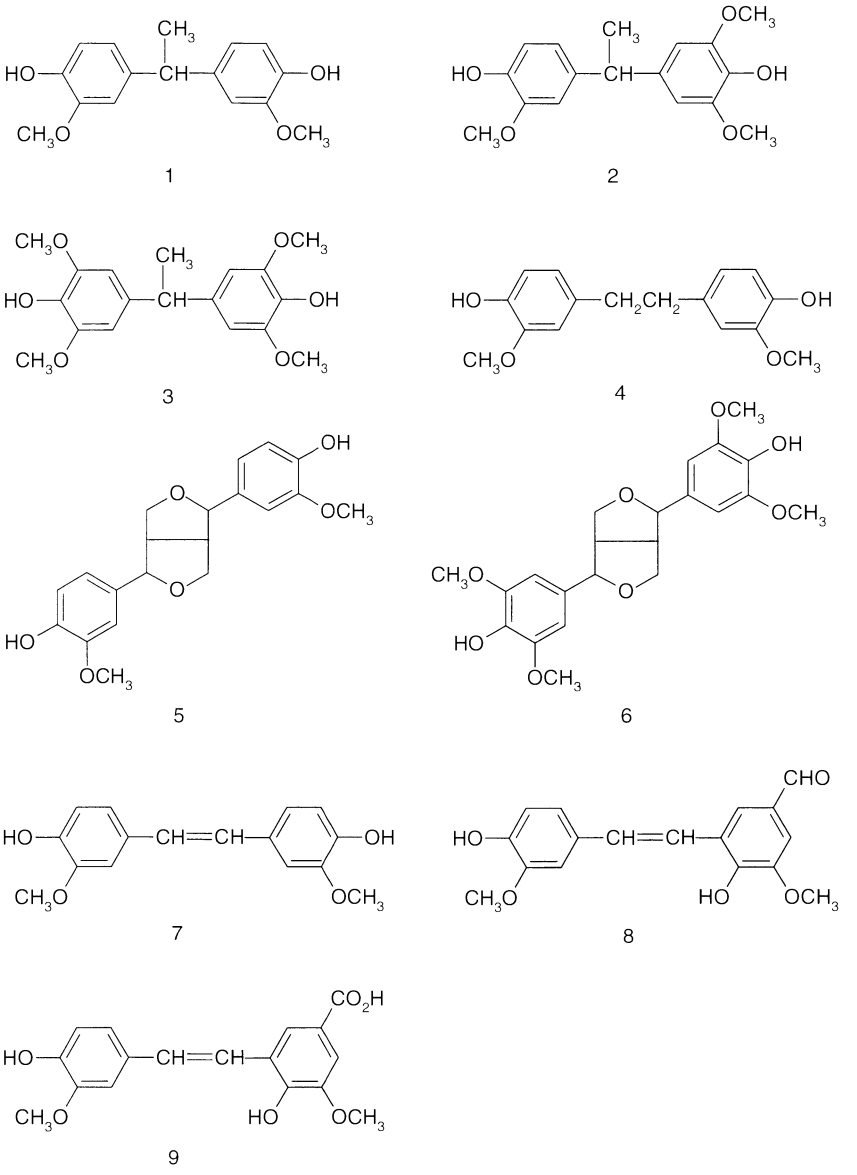

Fig. 7.6. Examples of identified dimeric lignin-derived compounds. **1** 1,1-diguaiacylethane; **2** 1-guaiacyl-1-syringylethane; **3** 1,1-disyringylethane; **4** 1,2-diguaiacylethane; **5** pinoresinol; **6** syringaresinol; **7** 4,4′-dihydroxy-3,3′-dimethoxystilbene; **8** 5-formyl-2,4′-dihydroxy-3,4′-dimethoxystilbene; and **9** 5-carboxy-2,4′-dihydroxy-3,4′-dimethoxystilbene

(*Tsuga*). Alkaline decomposition reactions of these materials are still poorly known, but for example hydroxymatairesinol has been shown by Ekman et al. (1979) to yield a butenoic acid derivative, (*E*)-4-guaiacyl-2-vanillyl-3-butenoic acid. Further work with pure lignans or isolated lignan fractions would apparently result in identification of several other novel transformation products.

It was mentioned above that black liquors contain comparatively large amounts of certain compounds, α-hydroxyarylalkanoic acids, that are formed as a result of condensation reactions between degradation intermediates of lignin and carbohydrates. Other types of lignin-derived condensation products occur in soda-anthraquinone (AQ) black liquors, i.e., those formed from AQ and lignin fragments. Although only a few such compounds have been isolated and identified in genuine liquors (Fullerton and Fleming 1980), model experiments have shown that a wide variety of them may actually exist. Their formation is obviously one of the main reasons for the partial consumption of AQ in the corresponding liquors.

Gas Chromatography and Mass Spectrometry. The choice of the GC conditions may depend on the derivatization method used and also on the desired depth of the analysis. As no comprehensive comparisons have yet been carried out, it is not reasonable to recommend any specific conditions. Instead, recent publications based on the use of capillary GC and trimethylsilyl derivatives can be of some assistance. Some recommended publications on mass spectral data have been compiled in Table 7.8.

Table 7.8. Selected references for mass spectra of lignin degradation products found or expected in black liquors

Compound type	Derivatives	References
Alkylphenols	None	Meier and Faix (1992)
Ketonic compounds	None	Meier and Faix (1992)
Ketonic compounds	TMS	Niemelä (1990b)
Sulfur-containing phenols	None	Niemelä (1990b)
Sulfur-containing phenols	TMS	Niemelä (1990b)
Carboxylic acids	TMS	Chapman et al. (1970), Löwendahl et al. (1978)
Catechols	TMS	Niemelä (1989a)
Stilbenes	None	Niemelä (1990b)
Stilbenes	Acetyl	Gierer and Lindeberg (1980)
Stilbenes	TMS	Niemelä (1993a)
Resinols	None	Van der Klashorst and Strauss (1987a)
Resinols	TMS	Niemelä (1990b)
Other dimers	Acetyl	Gierer and Lindeberg (1980), Lapierre et al. (1991)
Other dimers	TMS	Niemelä (1990b), Lapierre et al. (1991), Goñi (1992)

7.2.3 Extractives and Their Transformation Products

7.2.3.1 Fatty and Resin Acids and Terpenoids

Various extractives or their transformation products are present in black liquors. The extractives of commercial importance, in particular tall oil constituents, are usually analyzed in the isolated crude or distilled tall oils, as other types of compounds may interfere with their determination in the black liquors. In the widely used Saltsman-Kuiken (1959) method, the acidified black liquor is extracted with petroleum ether. Subsequently, the organic phase is usually washed with more polar solvents or with a water-acetone-methanol solution in order to remove more polar compounds. This procedure may thus give a fraction that differs from tall oil skimmings.

For more detailed characterization, various isolated fractions can be analyzed by GC and GC/MS. Neutral compounds, such as fatty alcohols, diterpene aldehydes, diterpene alcohols, triterpenoids, steroids, and polyprenols can be extracted from the alkaline liquors, although some fatty and resin acids will also be recovered. Many of these compounds can be analyzed by GC and GC/MS without any derivatization, but all the alcohols will be more easily detected if a proper derivatization, such as trimethylsilylation, is used. Under suitable chromatographic conditions, the detection of all typical polyprenols up to betulaprenol-9 is possible after trimethylsilylation (Niemelä 1990b). The most volatile extractives, monoterpenoids, can be analyzed from black liquors with head-space GC.

Fatty and resin acids, recovered from the acidified black liquor or tall oil skimmings, are usually analyzed by GC and GC/MS after methylation. Preparation of trimethylsilyl esters from the resin and fatty acids is not a widely used procedure, but trimethylsilylation after methylation can be used to enhance detection of hydroxy fatty acids and hydroxy resin acids that might be present.

7.2.3.2 Other Compounds

Various phenolic extractives, such as hydroxystilbenes, lignans, ellagitannins, flavonoids, polyphenols, or their reaction products, occur in black liquors. Their amounts and especially their nature depend on the wood material used. For example, various polyphenols are characteristic compounds of several tropical hardwoods. So far, only scattered attention has been paid to determine these constituents in pulping liquors, and thus analytical methods have not yet been firmly established.

All black liquors contain some glycerol, derived from the hydrolysis of glycerides. As a polar compound, glycerol can be detected in the polyhydroxy acid fraction. Other neutral compounds often present in the same fraction include

small amounts of pinitol, inositols, and other cyclic compounds. They are all most conveniently analyzed by GC as their TMS ethers.

7.2.4 Miscellaneous Compounds

All black liquors contain minor amounts of miscellaneous low-molecular weight compounds (Table 7.9). These may represent indigenous wood constituents, or they may be derived from proteins or suberin, or from reactions of elemental sulfur with various organic compounds. Some compounds can also represent impurities or contaminants entering the black liquor from several different sources. The impurities are more frequently found in industrial samples.

7.2.4.1 Protein-Derived Compounds

The behavior of nitrogen-containing substances during kraft cooking is receiving more attention, as control of NO_x-emissions from recovery furnaces is becoming more and more important. Currently, little is known about the nitrogen-bearing compounds in black liquors, although various extractions and other procedures have made it possible to isolate and identify several cyclic and straight-chain nitrogen compounds. A substantial amount of nitrogen is apparently liberated as ammonia and trimethylamine, but some nitrogen can even be bound to lignin precipitates.

Table 7.9. Some typical miscellaneous lipophilic compounds in kraft black liquors

Black liquor	Miscellaneous solvent-recoverable compounds
Alkaline (initial) liquor	Aliphatic hydrocarbons Aliphatic ketones and alcohols Alkyl-2-cyclopenten-1-ones, benzoquinones Dimethylpolysulfides, dithioethers, thiophenes Elemental sulfur Nitrogenous compounds
Subsequently acidified liquor	Alkyl-2-hydroxy-2-cyclopenten-1-ones Thiophenecarboxylic acids, methyl polysulfanes Nitrogenous compounds Long-chain dicarboxylic acids, hydroxy fatty acids

7.2.4.2 Suberin-Derived Compounds

Suberin-derived hydroxy fatty acids and long-chain dicarboxylic acids can be found in small amounts in many black liquors, particularly after pulping of inadequately debarked wood or whole wood. These compounds can be isolated from acidified black liquors by toluene extraction, after which they can be analyzed by GC and GC/MS as methyl ester trimethylsilyl ethers or as per(trimethylsilyl) derivatives (Kolattukudy 1980; Goñi and Hedges 1990; Goñi 1992).

7.2.4.3 Other Compounds

The extraction of alkaline kraft black liquors removes simple aliphatic ketones and alcohols, alkyl-2-cyclopenten-1-ones, benzoquinones, dimethylpolysulfides, dithioethers, and thiophenes, in addition to the previously-mentioned neutral extractives. Several solvents, such as chloroform, pentane, toluene, diethyl ether, and ethyl acetate can be used to isolate these compounds. The isolated and concentrated fractions should be analyzed by capillary GC and GC/MS before and after derivatization (preferentially trimethylsilylation).

Apart from certain extractives, the underivatized organic compounds in the alkaline extract include aliphatic ketones, benzoquinones, alkyl-2-cyclopenten-1-ones, and aliphatic sulfur compounds. Two neutral sulfides, dimethylsulfide and dimethyldisulfide, have traditionally been analyzed by GC under different conditions after extraction from alkaline liquors (Prakash and Murray 1976). The extension of the run times and the use of modified conditions have recently revealed that dimethylpolysulfides with up to five sulfur atoms can be detected in this way (Niemelä 1990b).

Other neutral aliphatic sulfur compounds, all recently discovered, include some dithioethers and several alkyl-, acyl-, and hydroxyalkyl-substituted thiophenes (Niemelä 1990b). The latter compounds are more easily detected if the hydroxy groups are derivatized prior to the GC runs. Although all these compounds belong to minor black liquor compounds, they are of certain interest as they have revealed some new features of the reactions of elemental sulfur or sodium sulfide during kraft pulping. Elemental sulfur can also be found in the alkaline extracts. The GC/MS studies have shown that cyclohexasulfur and cycloheptasulfur are also present, in addition to cyclooctasulfur (Seefelt and Dimmel 1983; Niemelä 1989d, 1990b).

Apart from the acidic extractives, the major miscellaneous compounds in the subsequent extract of the acidified kraft black liquor are alkyl-substituted 2-hydroxy-2-cyclopenten-1-ones (Niemelä 1988c, 1989e; Berthold and Gellerstedt 1993), but numerous other compounds, such as thiophenecarboxylic acids and methyl polysulfanes (Niemelä 1990b), have also been detect-

ed and identified. As early as in 1954, Enkvist et al. were able to isolate and identify 2-hydroxy-3-methyl-2-cyclopenten-1-one in a kraft black liquor, but only recently several further compounds of this type in black liquors have been detected. Examples of the compounds identified are given in Fig. 7.7. The most suitable solvents for their isolation include chloroform and toluene. The subsequent GC and GC/MS analysis should be carried out both with and without derivatization. The analysis of the underivatized compounds allows MS identification, as the mass spectra of the different isomers usually differ. However, the supporting GC/MS analysis and extracted ion chromatography of the trimethylsilylated extract allows a thorough preliminary screening of these compounds as their presence is readily indicated by characteristic, abundant $[M\text{-}15]^+$ ions. Several experiments with model compounds have demonstrated that the 2-hydroxy-2-cyclopenten-1-ones are derived from carbohydrates (Niemelä 1988c, 1989e; Nishimura and Mihara 1990).

The main aliphatic sulfur compounds in this fraction include methanethiol (methyl mercaptan), methyl polysulfanes, and thiophenecarboxylic acids. Several GC methods have been reported for their determination.

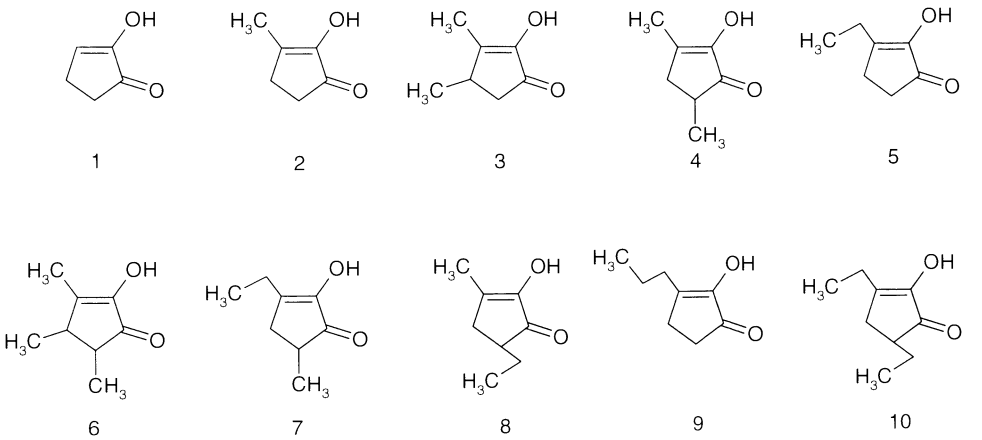

Fig. 7.7. Examples of 2-hydroxy-2-cyclopenten-1-ones found in black liquors. **1** 2-hydroxy-2-cyclopenten-1-one; **2** 2-hydroxy-3-methyl-2-cyclopenten-1-one; **3** 2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one; **4** 2-hydroxy-3,5-dimethyl-2-cyclopenten-1-one; **5** 3-ethyl-2-hydroxy-2-cyclopenten-1-one; **6** 2-hydroxy-3,4,5-trimethyl-2-cyclopenten-1-one; **7** 3-ethyl-2-hydroxy-5-methyl-2-cyclopenten-1-one; **8** 5-ethyl-2-hydroxy-3-methyl-2-cyclopenten-1-one; **9** 2-hydroxy-3-propyl-2-cyclopenten-1-one; and **10** 3,5-diethyl-2-hydroxy-2-cyclopenten-1-one

7.3 Sulfite Spent Liquors

The importance of sulfite pulping has clearly decreased during recent decades, and nowadays less than 20% of chemical pulp is produced by this method. Consequently, general interest in chemical composition of the sulfite spent liquors (SSLs) has likewise decreased. This is shown, for example, by reluctance to adopt and further refine modern analytical methods for the detailed analysis of the composition of these liquors. Thus, most of our knowledge on the composition of the sulfite spent liquors dates from the 1950s, 1960s, and 1970s.

As already discussed in Section 7.1, typical sulfite spent liquors differ from alkaline pulping liquors (Table 7.1) in many respects. More detailed examples of typical SSL compositions are provided in Table 7.10.

In this section, main attention is focused on the analysis of liquors from acid sulfite pulping, but some references to alkaline or neutral (high-yield) sulfite pulping processes are also given. Nevertheless, many compounds in such liquors can be analyzed by using or slightly modifying the methods described in Section 7.2.

Many constituents, such as polysaccharides, extractives, and simple phenolic compounds present in the sulfite spent liquors can be analyzed by the methods already discussed and these will not be repeated here. Instead, the discussion below is mainly focused on the compounds most characteristic of the acid sulfite liquors.

Table 7.10. Examples of the composition of spent sulfite liquors (Sjöström 1993), given as kg per ton pulp

Component	Spruce	Birch
Lignosulfonates[a]	480	370
M > 5000 Da	245	55
Carbohydrates[b]	280	375
Arabinose	10	10
Xylose	60	340
Mannose	120	10
Galactose	50	10
Glucose	40	5
Aldonic acids	50	95
Acetic acid	40	100
Extractives	40	40
Other compounds	40	60

[a] Calculated as lignin.
[b] Monosaccharides form 80–85% of carbohydrates, the rest is oligo- and polysaccharides.

7.3.1 Degradation Products of Polysaccharides

The main polysaccharide-derived low-molecular weight compounds in sulfite spent liquors include oligo- and monosaccharides, aldonic acids, and acetic acid, this last being a well-established deacetylation product of *O*-acetylated hemicelluloses. In addition, a wide variety of miscellaneous other dehydration, degradation, and sulfonation products may exist.

Various chromatographic methods are available for the isolation and determination of mono- and oligosaccharides in the sulfite spent liquors (Samuelson and Simonson 1962; Pearl and Beyer 1964a,b; Pettersson and Samuelson 1967; Pfister and Sjöström 1977). In routine applications, the neutral carbohydrates can be separated from aldonic and uronic acids by ion-exchange chromatography (IEC) (Pfister and Sjöström 1977), after which both of the fractions can be analyzed as their TMS derivatives by GC. The aldonic acids can be analyzed either as their lactones, or alternatively, they can be converted to ammonium salts before the trimethylsilylation. The main advantage of the latter option is generation of only one GC peak from each aldonic acid, instead of the formation of both of the 1,4- and 1,5-lactones from pentonic and hexonic acids. For more detailed investigations of SSL carbohydrates, such as for characterization of oligomeric uronic acids, Pettersson and Samuelson (1967) have developed LC fractionation methods based on the use of anion exchange resins.

There is some evidence that minor amounts of partially acetylated carbohydrates may also survive the pulping conditions (Croon 1962), but no routine methods have yet been refined for their determination.

Various sulfonated, carbohydrate-derived carboxylic acids are also evidently present in sulfite spent liquors (Yllner 1956; Hardell and Theander 1965), albeit probably in relatively small concentrations. Such compounds are known to form per(trimethylsilyl) derivatives (Palomo and Cabré 1987), and thus they could be analyzed by GC/MS. The lack of published MS reference data requires, however, substantial supporting work with known model compounds. Probably for these reasons, the aliphatic sulfonic acids have received only limited interest so far.

Other carbohydrate-derived compounds include simple carboxylic acids and carbonyl compounds, formed as a result of dehydration reactions. Examples of such compounds include acetaldehyde, pyruvaldehyde, 5-hydroxymethylfurfural, and pyruvic acid. Various chromatographic techniques, such as IEC (Christofferson 1967) can be used for their determination.

7.3.2 Lignosulfonates and Their Degradation Products

High-molecular weight lignosulfonates form the main organic fraction of the sulfite spent liquors (Table 7.10). They can be separated from the low-

molecular weight lignosulfonates and other low-molecular weight acids by using anion exchange resins (Sjöström et al. 1963) or dialysis membranes (Sjöström and Haglund 1964). For further investigations, the lignosulfonates can be isolated and fractionated with ultrafiltration or through complexing with alkylamines (Kontturi and Sundholm 1986). The cited papers contain detailed descriptions for the determination of the concentrations of lignosulfonates by UV spectrophotometry, degree of sulfonation, acid amount by potentiometric tritration, and other relevant data.

A large number of low-molecular weight aromatic compounds apparently occur in SSLs, although these have never been studied to the same extent as those present in kraft pulping liquors. With the help of classical chromatographic methods, a number of monomeric and dimeric compounds have been isolated and identified in various sulfite spent liquors. The monomeric compounds typically include vanillin, syringaldehyde, syringol, vanillic acid, syringic acid, 4-hydroxybenzoic acid, acetovanillone, acetosyringone, and dihydroconiferyl alcohol (Kvasnicka and McLaughlin 1955; Pearl and Beyer 1961, 1964a,b).

The isolated and identified dimeric compounds include 4,4′-dihydroxy-3,3′-dimethoxystilbene, syringaresinol, and α-conidendrin (sulfite liquor lactone) – the latter compound has been found even in hardwood spent liquors (Pearl 1958; Pearl and Beyer 1961).

Moreover, several sulfonated monomeric compounds have been isolated and identified by classical methods, for example by Parrish (1964, 1967). Some examples of sulfonated and nonsulfonated aromatic sulfite spent liquor compounds are given in Fig. 7.8.

More recently, a large number of sulfonated lignin-derived monomers and dimers have been isolated from neutral sulfite pulping liquors by HPLC (Luthe 1990). Before the chromatographic separation, the sulfonates were converted to free acids, extracted with butanol, and methylated. The structures of the isolated and purified compounds were finally determined with NMR spectroscopy and mass spectrometry. Altogether, this makes it reasonable to believe that several further sulfonated and nonsulfonated aromatics are present in most sulfite spent liquors, awaiting further identification. The application of modern GC and GC/MS methods (Sect. 7.2.4), accompanied by chromatographic isolations and NMR studies, would undoubtedly result in more comprehensive knowledge on aromatic compounds in acidic and in other SSLs (Hachey et al. 1986; Sakai et al. 1986). As shown by Dahlman and Månsson (1996), capillary zone electrophoresis may also be successfully applied for the determination of low-molecular weight lignosulfonates.

7.3.3 Extractives

The determination of various extractives in SSLs has never received the same status as in kraft black liquors. This is understandable as *p*-cymene (sulfite tur-

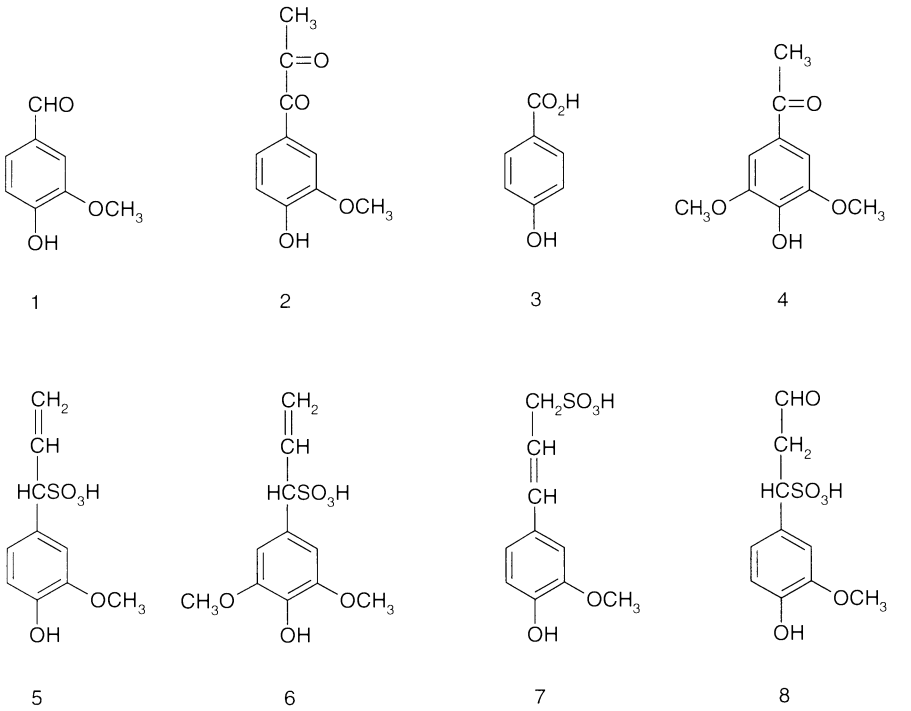

Fig. 7.8. Examples of aromatic spent sulfite liquor compounds. **1** vanillin; **2** 1-guaiacyl-1,2-propanedione; **3** 4-hydroxybenzoic acid; **4** acetosyringone; **5** 1-guaiacyl-2-propene-1-sulfonic acid; **6** 1-syringyl-2-propene-1-sulfonic acid; **7** 3-guaiacyl-2-propene-1-sulfonic acid; and **8** 3-guiacylpropanal-3-sulfonic acid

pentine) has traditionally been the sole extractives-derived by-product from acidic sulfite pulping. Thus, typical extractives, such as fatty acids, fatty alcohols, sterols, and lignans have only relatively seldom been analyzed in any SSL samples (e.g., Pearl et al. 1958; Pearl and McCoy 1961). In these cases, classical isolation and characterization methods have been used, but obviously modern analytical tools would offer more powerful alternatives for further investigations.

7.4 Inorganic Components

Quantitative and detailed data on the composition of the inorganic fraction in the cooking liquors are of importance in many respects, particularly when predicting the combustion behavior of various spent liquors in the recovery furnace. Because the composition of the inorganics vary greatly, depending on

the cooking method and the wood material, an exhaustive discussion of the topic is not possible in the limited space of the following text, but references to the relevant literature are given below.

7.4.1 Kraft Black Liquors

The white liquor is mainly composed of sodium hydroxide (NaOH) and sodium sulfide (Na_2S) in addition to some sodium carbonate (Na_2CO_3) and sodium salts of oxidized sulfur-containing anions (Rydholm 1965; Wilson 1968; Clayton et al. 1989; Grace 1989; Mimms et al. 1993). According to the terminology, the active alkali (NaOH + Na_2S) and effective alkali (NaOH + 1/2 Na_2S) are calculated as sodium equivalents and the concentration of the liquor is expressed as grams of NaOH (predominantly in Europe) or Na_2O (predominantly in North America) per liter of solution. In modern pulping chemistry, molar units are often used instead of weight units. Both active and effective alkali can also be expressed as a percentage of dry-wood weight. The alkali of the white liquor charged to the digester is consumed during cooking for neutralization of the organic acids which are primarily formed in the degradation reactions of wood carbohydrates resulting in the decrease of the pH. The composition of the active inorganic species (HS^-, S^{2-}, and HO^-) can be followed from the equilibrium diagrams (the Bjerrum diagrams) valid in the system (Rydholm 1965; Sjöström 1993).

The inactive inorganic fraction of the black liquor consists of sodium carbonate and various conversion products of sodium sulfide, mainly sodium sulfate (Na_2SO_4), sodium thiosulfate ($Na_2S_2O_3$), and sodium sulfite (Na_2SO_3) (Rydholm 1965; Clayton et al. 1989; Grace 1989; Mimms et al. 1993). In addition, various inorganic cations and anions are present, originating from the wood material (Chap. 10), equipment, and process water. Accumulation of these inactive dead-load chemicals increases the load on the recovery furnace, and can cause scaling in the digester and especially in the subsequent evaporation of black liquor. In this respect, silicates and calcium salts are the most harmful components. However, silicates are not abundant in usual wood raw materials, but occur in large amounts in different non-wood raw materials such as cereal straw (deposited as silica crystals). Accumulation of chlorides can lead to corrosion problems. The sticky range in the recovery furnace depends mainly on the chloride and potassium content of the entrained solids.

Table 7.11 gives an example of the composition of the inorganic fraction of the black liquor (only major components are considered). Because of the presence of a large number of inorganic components, some of them occurring in trace amounts, a comprehensive analysis is complicated and not suitable for routine purposes. In addition, sodium and sulfur are both bound to the organic and inorganic fractions of the black liquor.

Table 7.11. Examples of the analysis data on pine kraft black liquor

Compound, % of the total inorganics		Element, % of the dry solids	
Na_2S	17	Sodium[a]	19.1
Na_2SO_4	12	Sulfur	4.8
$Na_2S_2O_3$	14	Potassium	0.9
Na_2SO_3	7	Chlorine	0.2
NaOH	6	Carbon	38.0
Na_2CO_3	32	Oxygen	33.1
Others[b]	12	Hydrogen	3.6
		Nitrogen	0.1
		Others[c]	0.2

[a] About 65% of sodium is bound to organic material.
[b] Including mainly potassium salts and chlorides. The sodium and sulfur bound to organics are not included.
[c] Small amounts of Si, Ca, Fe, Al, Mg, Mn, and P are also present.

The commonly used methods for analyzing black liquor inorganics are mainly based on conventional wet chemistry, although a gradual transition from the old gravimetric and titrimetric procedures to the more sophisticated techniques, such as IC, polarography, CE, and different spectrometric determinations, is occurring (Clayton et al. 1989; Douek et al. 1993; Milanova and Dorris 1994; Ulmgren et al. 1994). In most cases, there are alternative ways of performing the procedure for each determination. Examples of the common methods are given in Table 7.12. Many of these methods have been proposed as national standards. Besides the methods for the black liquor there are several standard methods (e.g., TAPPI T 624cm–85 and CPPA J.12) for analyzing inorganic components in the white and green liquor of the kraft mill's recovery cycle (Paulonis and Krishnagopalan 1994a,b).

7.4.2 Sulfite Spent Liquors

There are a number of modifications of conventional acid calcium sulfite cooking process. In modern sulfite cooking, mainly sodium and magnesium are used, and the inorganic chemicals are recovered and regenerated. The use of sodium makes it possible to choose almost arbitrary pH conditions.

The active species in the sulfite process are sulfur dioxide (SO_2), hydrogen sulfite (HSO_3^-), and sulfite (SO_3^{2-}) varying in their proportions on the actual pH of the cooking liquor (Rydholm 1965; Ingruber 1985; Sjöström 1993). Their proportions can be calculated on the basis of the dissociation constants for sulfurous acid (H_2SO_3) or simply from the standard titration curve. The active base is the cation bound to the hydrogen sulfite and sulfite ions and its con-

Table 7.12. General standard methods for analyzing black liquor inorganics

Determination	Method[a]
Inorganic/organic ratio	TAPPI T 625 cm-85, KCL 61:83
Total, active, and effective alkali	TAPPI T 625 cm-85, CPPA J.15P, KCL 67a:87
Residual alkali (hydroxide ion)	SCAN-N 33:94, KCL 67:87
Ash	CPPA J.15P, KCL 59:83
Sulfated ash	TAPPI 625 cm-85, KCL 60:64
Sulfide and hydrogen sulfide	TAPPI T 625 cm-85 and 699 om-87, SCAN-N 31:94, KCL 69:91
Sulfate	TAPPI T 625 cm-85 and 699 om-87, KCL 71:81
Sulfite and thiosulfate	TAPPI T 625 cm-85 and 699 om-87, KCL 70:83
Polysulfide sulfur	TAPPI T 625 cm-85, CPPA J.15P, KCL 72a:84
Carbonate	TAPPI T 699 om-87, KCL 68:91
Chloride	TAPPI T 699 om-87
Sodium	TAPPI T 623 cm-83 and 625 cm-85, KCL 65:82
Sulfur	TAPPI T 625 cm-85, CPPA J.15P, KCL 63:64 and 63a:84
Carbon and hydrogen	KCL 62:64
Nitrogen	KCL 64:92
Chlorine	KCL 73a:84
Silica, iron, aluminum, calcium, magnesium, manganese, potassium, and phosphorous	TAPPI T 625 cm-85, CPPA J.15P, KCL 231:92, STFI AH41-1:83, AH41-3:83, AH41-4:81, AH41-7:80, AH41-8:80, and AH42-5:81

[a] TAPPI, The Technical Association of Pulp and Paper Industry; CPPA, The Canadian Pulp and Paper Association; SCAN The Scandinavian Pulp, Paper, and Board Testing Committee; KCL, The Finnish Pulp and Paper Research Institute; STFI, The Swedish Pulp and Paper Research Institute.

centration is usually expressed as the grams of Na_2O per liter of solution. Naturally in cooking liquors of higher pH, an excess of alkali is present.

According to the old terminology, the total amount of sulfur dioxide present in cooking acid or cooking liquor is divided into so-called free and combined sulfur dioxide (Ingruber 1985). The concentrations of total, free, and combined sulfur dioxide are expressed as the grams of SO_2 per 100 ml of solution. The standard determinations (TAPPI T 604 cm-85, CPPA J.1H, CPPA J.13P) are originally based on the Palmrose method (Palmrose 1935): titration with potassium iodate for the total SO_2 and titration with sodium hydroxide for the free SO_2. If the liquor contains lignin derivatives (e.g., relief liquor) where loosely combined or reversible SO_2 is associated with the organic matter, the determination requires corrective procedures.

As in the kraft pulping process, but depending on different reactions, the active cooking chemicals are consumed and inactivated during sulfite pulping. Simple titration methods used for the fresh cooking liquor are not directly applicable because of the presence of interfering weak acids (cleavage and conversion products of wood hemicelluloses). Such cases are, for example, the

liquor samples withdrawn from the first stage of a two-stage cook as well as the liquors from semichemical and chemimechanical pulping. The remaining active cooking chemicals or bound sulfur dioxide can be calculated on the basis of iodometric titration of the total amount of sulfur dioxide and the liquor pH (Sjöström et al. 1963).

Thiosulfates and polythionates are harmful components generated during pulping and recovery of cooking chemicals. Standard procedures are available for their determination. For the analysis of other inorganic components (elementary composition, etc.), see also Chapter 10.

7.5 Physical Properties

The fluidity and other physical properties of spent liquors at a constant temperature and pressure are related both to the concentration and to the composition of the dissolved solids. The respective relationships are complex, but they play a key role in the evaporation and combustion behavior of the spent liquors (Venkatesh and Nguyen 1985; Adams and Frederick 1988). In addition, the physical properties of spent liquors vary considerably during mill operations, and they also differ from mill to mill. Although a number of standard methods are available, extensive research is still needed for developing adequate methods for testing the physical properties of spent liquors.

Mainly the kraft black liquors are briefly considered in the following presentation, but, in principle, the methods illuminated here are applicable to other spent liquors as well.

7.5.1 Dry Solids Content

According to the standard methods (TAPPI T 650 om-89, CPPA J.15P) the dry solids content is determined by drying the liquor sample at 105 °C in a forced air oven to a constant weight. However, the values obtained are somewhat too high, due to the oxidation of sulfide to thiosulfate (Söderhjelm and Sågfors 1994a). This error (normally less than 4%), which is larger at high dry solids and sulfide contents, can be at least partly eliminated when the initial content of sodium sulfide is known. Other methods include the determination of the water content by azeotropic toluene distillation and vacuum drying (Adams and Frederick 1988; Grace 1989). The calculated dry solids contents obtained by these methods have been found to be close to those from the standard oven-drying procedures.

7.5.2 Density

At low solids contents, the density of black liquor is close to that of water at the same temperature. At higher solids contents, the density depends on the composition of the dissolved material, especially inorganics (Adams and Frederick 1988; Grace 1989; Zaman et al. 1994). In practice, the density of black liquor can be measured with a hydrometer that is calibrated in degrees of Baumé. The density is reported as specific gravity referred to the density of water at a reference temperature, usually 16 °C (60 °F). Hydrometer readings (°Bé) taken at any other temperature should be corrected for temperature based on the data for each respective liquor. The calculation is made using the following relationship: Specific gravity = 145/(145–°Bé).

The density characteristics of various black liquors are almost similar, and the specific gravity is nearly a linear function of the dry solids content. A slight deviation from this linearity begins above 25%, and it then successively becomes more pronounced. In this case, temperature effects are minor compared with the effect of changes in solids content.

7.5.3 Viscosity

The rheological properties of spent liquors are important in several respects (Söderhjelm 1986, 1988; Ramamurthy et al. 1993; Söderhjelm and Sågfors 1994b; Zaman and Fricke 1995). For example, viscosity affects heat transfer during evaporation, the capacity of pumps, the formation of liquor droplets in the recovery furnace spray, etc. Viscosity (internal resistance) is defined as the ratio of the shearing stress to the shear rate for a fluid subjected to a shearing force. There are no standard methods available for the determination of viscosity in kraft black liquors. The dynamic viscosity (expressed as mPa·s) is usually measured by applying rotational (parallel-plate, cone-and-plate, and concentric cylinder viscometers) or tube viscometers. In general, when the solids content is below 50%, the black liquors behave as a Newtonian fluid (viscosity is independent of the shear rate) but becomes thixotropic (non-Newtonian) at higher dry solids contents (usually above 65%). The viscosity is not only related to the temperature and solids content, but the liquor composition has a marked influence as well. For example, liquors with a low content of residual alkali or a high content of polysaccharides such as xylans show high viscosities.

7.5.4 Heating Value

The gross (or higher) heating value (HHV) of black liquors can be determined calorimetrically using an excess of oxygen according to the standard method of TAPPI T 684 om-90. HHV can also be estimated on the basis of dry solids composition and is roughly equal to the sum of the heating values of individual component groups (lignin, carbohydrate-derived material, and extractives) and components (mainly inorganic compounds) (Adams and Frederick 1988; Grace 1989). Of the inorganic matter, the reduced sulfur (e.g., sodium sulfide and sodium thiosulfate) increases the HHV, whereas the other inorganics acting as diluents decrease it. Typical HHVs of kraft black liquors are in the range 13.5–15.5 MJ/kg of black liquor solids. An actual energy quantity available in the recovery furnace can be calculated from the HHV. This calculated number is the net heating value (NHV), often referred to as the lower heating value (LHV) and is usually 20–25% less than indicated by the HHV.

7.5.5 Other Physical Parameters

In addition to the determinations described above, several other physical properties of black liquors can be measured by specific (non-standardized) methods (Adams and Frederick 1988; Grace 1989; Ramamurthy et al. 1993; Stoy and Fricke 1994). For example, the vapor pressure of black liquor is an important measure of the liquor's boiling characteristics in evaporators. It can be measured in terms of vapor pressure lowering or boiling point rise (BPR). Surface tension is determined for estimating the droplet formation during spraying of the liquor into the recovery furnace. The data on thermal conductivity and heat capacity are used in calculations of the heat transfer rates in evaporators.

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8 Analysis of Bleach Liquors

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8.1 Introduction

Since the early 1970s, great efforts have been made in developing suitable analytical methods for the chemical characterization of the organic material in bleach liquors from kraft and sulfite pulp mills. Several different analytical methods have been needed, since this material is composed of a very complex mixture of substances varying in chemical structure and molecular weight. The interest has particularly been focused on methods for monitoring the formation and discharge of chlorinated organic matter. Methods for determination of both sum parameters and specific compounds have been developed for this purpose.

During recent years, the most important objective in bleaching technology has been to minimize the pollution load in order to satisfy stringent environmental needs. In this connection, it has been necessary to develop more sensitive methods for the analysis of bleach liquors. In modern bleach plants, the use of elemental chlorine has been drastically reduced or totally eliminated, and for that reason additional methods for monitoring and detection of trace amounts of chlorinated compounds have been necessary. Another reason has been the introduction of totally chlorine-free bleaching processes (TCF bleaching) in which chlorine dioxide is also eliminated, and instead only chlorine-free chemicals, mainly oxygen, ozone, and hydrogen peroxide, are used. There has therefore been a need for creating new or improved methods for the determination of nonchlorinated organic compounds.

This chapter deals with different analytical methods that have been found to be useful for characterizing bleach liquors and effluents from chlorine bleaching, elemental chlorine-free (ECF) bleaching, and TCF bleaching. The intention has been not only to provide the reader with a series of analytical procedures and references, but also to give a brief description of the background of the recommended methods.

8.2 Methods for Determination of Sum Parameters

8.2.1 Adsorbable Organic Halogens (AOX)

The amount of organically bound chlorine in bleach liquors can be determined by several methods. The easiest way is to calculate it as the difference between

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the total amount of chlorine and the amount present as inorganic chlorides ("difference method"). However, in the case of chlorine bleaching, inorganic chlorides occur in quantities that are an order of magnitude greater than the amount of organically bound chlorine, which makes the difference method inaccurate.

A more precise determination can be accomplished by measuring the total organic chlorine (TOCl). In this method (Sjöström et al. 1982), the inorganic chlorides are first removed, and the organically bound chlorine is then determined using Schöniger combustion followed by titrimetric determination of the hydrogen chloride formed during combustion. However, removal of inorganic chlorides from the bleach liquor sample is a rather laborious procedure, including both ultrafiltration and adsorption on a polymer resin (XAD resin).

The values obtained by the methods mentioned above do not adequately consider the organically bound chlorine in volatile chlorinated compounds, since such compounds tend to escape during the sample treatment. Furthermore, these methods do not have the sensitivity required for analysis of liquors from modern ECF bleaching. Therefore, these methods have in many laboratories been replaced by the AOX method, which is more sensitive, accurate, and less laborious.

In the AOX method (SCAN test 1989), the chlorinated organic material in the sample is first adsorbed on activated carbon, which is then washed with a nitrate solution in order to remove any residues of inorganic chlorine compounds. The activated carbon, including the adherent chlorinated organic material, is quantitatively combusted with oxygen in a quartz tube at high temperature (about 1000 °C). The hydrogen chloride formed during the combustion step is adsorbed in an electrolyte solution and determined by microcoulometric titration.

Volatile chlorinated compounds are in most cases adsorbed on the activated carbon and are thus accounted for by this method. However, highly hydrophilic chlorinated compounds, e.g., chlorinated acetic acids, may be only partly adsorbed onto the carbon.

Several procedures have been proposed for adsorption of chlorinated organic material on the activated carbon in the AOX method (Fraser and Reeve 1989). The two most commonly used techniques are either contact adsorption through mixing the sample with activated carbon or column adsorption using a short column packed with the carbon. These two adsorption procedures may give slightly different results. However, for most purposes the agreement is quite sufficient for accurate determination of AOX in bleach liquors.

In summary, both the contact adsorption and column adsorption versions of the AOX method are quite rapid and reliable methods for determination of the organically bound chlorine in bleach liquors. The instrumentation required is commercially available from several manufacturers.

8.2.2 Extractable Organic Halogens (EOX)

The extractable organically bound chlorine in bleach liquors is a subfraction of the total amount of organically bound chlorine as determined by the AOX method. The EOX value can be regarded as an estimate of the lipophilic part of the organically bound chlorine in bleach liquors.

For bleach liquors from chlorine and chlorine dioxide bleaching of kraft pulps, the EOX value is usually only a few percents of the corresponding AOX value, whereas in bleach liquors from sulfite pulp mills, the EOX value may constitute a larger fraction of the AOX.

Several procedures have been proposed for the determination of EOX. The main principle of all EOX methods is, however, extraction of the liquor sample with a nonpolar organic solvent (in most cases cyclohexane or *n*-hexane), followed by determination of the organically bound chlorine in the extract obtained. The extraction is carried out two to three times. It should be noted that the liquor sample must be acidified (pH 3) before extraction. Acidification is necessary in order to liberate all the acidic groups and to maximize the extraction yield.

Neutron activation analysis (NAA) has been suggested for the determination of organically bound chlorine in the extract obtained (Martinsen et al. 1988). However, NAA can only be carried out by a few specialized laboratories. A more practical way to determine the amount of organically bound chlorine in the extract is to use a technique similar to that applied in the AOX method (Lopez et al. 1994). After removal of the solvent by careful evaporation, the sample is combusted and the hydrogen chloride formed upon combustion is determined by microcoulorimetric titration. For the combustion step, and the subsequent microcoulorimetric determination, the same equipment as in the AOX method can be used.

8.2.3 Chemical Oxygen Demand (COD), Total Organic Carbon (TOC), and Biochemical Oxygen Demand (BOD)

The total amount of organic material in bleach liquors can be estimated by two different methods, COD and TOC. The BOD method can be used to estimate the amount of easily biodegradable organic material. COD and BOD are proposed as national standard methods by authorities in a number of countries. In addition, the Canadian Pulp and Paper Association (CPPA 1991, 1993) and the Scandinavian Pulp, Paper and Board Testing Committee (SCAN test 1971) have proposed their own standard methods. It is recommended that these standard procedures be followed in the countries where they have been developed.

COD. In the determination of COD, the oxidation reagent used is either $K_2Cr_2O_7$ (COD_{Cr}) or $KMnO_4$ (COD_{Mn}). The former reagent is more commonly used, since it gives a complete oxidation of the organic material. The sample is mixed with an excess of the reagent and the solution is heated. After a certain time, the amount of residual reagent is determined by titration or determined photometrically.

Under the conditions used in COD determinations, the organic material is oxidized according to the general formula:

$$CH_xO_y + (2 + x/2 - y)O \rightarrow CO_2 + x/2\,H_2O. \tag{8.1}$$

The oxidation reagents react according to Eqs. (8.2) and (8.3):

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O, \tag{8.2}$$

$$MnO_4^- + 8H^+ + 5e^- \rightarrow Mn^{2+} + 4H_2O. \tag{8.3}$$

It should be noted that organically bound sulfur as well as some inorganic ions (e.g., Fe^{2+}, S^{2-}, and Cl^-) are also oxidized, and are thus contributing to the total oxygen consumption. In samples with high chloride concentration, mercury salts can be added to prevent interference from the chloride ions.

TOC. The TOC analysis is based on oxidation of organically bound carbon to carbon dioxide. TOC can be determined either by chemical or thermal oxidation. In the former case, the sample is chemically oxidized in a reactor with potassium peroxydisulfate, often under UV irradiation. In the thermal oxidation method, the sample is catalytically oxidized by oxygen or air at 700–900 °C.

For determination of the total carbon dioxide formed during the oxidation, a nondispersive infrared (NDIR) detector is commonly used. After separate measurement of the carbon dioxide originating from inorganic compounds (e.g., carbonates), the TOC is calculated as the difference between total carbon dioxide and carbon dioxide originating from inorganic sources.

BOD. The BOD method provides an estimation of the quantity of biologically degradable organic matter. The basic principle of this method is that certain microorganisms, which are added to the sample, decompose parts of the dissolved and suspended organic matter with consumption of oxygen. The BOD is calculated from measurements of the concentrations of the dissolved oxygen (DO) before and after a certain incubation period. The result is reported as BOD. In the Nordic countries, BOD_7 (7 days incubation) is used instead of the more commonly used BOD_5 (5 days incubation).

It is important to keep in mind that only a part of the biodegradable organic material in a bleach liquor is biochemically oxidized to carbon dioxide and water under the conditions used in the BOD method. Other parts are either partly oxidized (chemically changed) or consumed by the microorganisms

(transformed into new cell substance). As a result, the oxygen consumption observed in BOD tests is normally considerably lower than the theoretical oxygen demand.

8.3 Molecular Weight Distribution

The molecular weight distributions of the dissolved organic materials in bleach liquors (which are mainly lignin-derived fragments) are usually studied by high-performance size exclusion chromatography (HPSEC). Separation of organic molecules according to molecular size takes place in solution on columns filled with a rigid, porous packing. Small molecules penetrate the pores of the packing material more easily than larger molecules and will therefore appear later in the chromatogram.

In HPSEC analysis of the dissolved organic material in bleach liquors, aqueous systems using a buffer solution as mobile phase are normally preferred since this technique permits a direct analysis of the liquor without any sample preparation other than filtration. HPSEC techniques using organic solvents such as tetrahydrofuran as the mobile phase are more laborious and less useful since isolation of the organic material from the liquor (and sometimes also derivatization) is necessary prior to analysis.

Since bleach liquors also contain inorganic components, the choice of detectors in aqueous HPSEC is limited to those that are insensitive toward inorganic ions (e.g., metal ions, chloride, chlorate, and carbonate) present in these liquors. Normally, a UV detector operating at 270–280 nm is used. However, when isolated organic materials from bleach liquors (for example isolated high-molecular weight materials) are studied, refractive index (RI) detection can be used. RI detection may produce a more accurate molecular weight distribution when significant amounts of polysaccharides are present in the sample.

The HPSEC system must be calibrated in order to obtain a correlation between the retention time and the molecular weight. In aqueous HPSEC, narrow and well characterized polystyrene sulfonate fractions are commonly used as calibration standards. An exact correlation between retention time and molecular weight requires that the calibration standards and the analyzed sample have the same chemical structure. This requirement is, however, difficult to meet in HPSEC analysis of organic materials in bleach liquors because of the heterogeneous composition of this material. A possible association of molecules is also a further source of errors.

Molecular weight averages such as the number-average molecular weight ($\overline{M}_n$) and the weight-average molecular weight ($\overline{M}_w$) can be calculated from HPSEC data. However, in the case of the dissolved organic material in bleach liquors, these averages are of rather limited value since they are relative values with respect to the calibration substance used.

Figure 8.1 shows, as an example, the results from aqueous HPSEC analysis of two comparable effluents from a kraft mill with production of bleached softwood and hardwood pulps. It should be observed that the molecular weight scale on the X-axis is relative with respect to the calibration substance used (polystyrene sulfonate). As can be seen from the figure, the UV-absorbing organic material in the hardwood-derived effluent exhibited a lower apparent molecular weight than the corresponding material in the softwood-derived effluent.

In summary, aqueous HPSEC with UV detection is a rapid and useful method for comparisons between the apparent molecular weight distributions of the UV-absorbing (mainly lignin-derived) organic materials in different types of bleach liquors. It should, however, be kept in mind that dissolved carbohydrates without UV-absorbing groups will not be detected. It is also important to keep in mind that molecular weight-correlated plots such as the one in Fig. 8.1 do not give exact molecular weights, as discussed above. The molecular weight scale in such plots can, when calibrated with commercially available molecular weight standards such as polystyrene sulfonates, only be used for a rough estimation of the molecular weight region in which the investigated materials appear.

8.4 Characterization of Low-Molecular Weight Compounds

Great efforts have been made in a number of investigations during the past 20 years to identify various types of low-molecular weight organic compounds

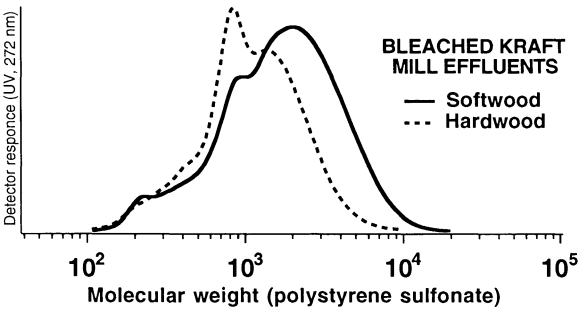

Fig. 8.1. Apparent molecular weight distributions of the UV-absorbing organic materials in whole mill effluents from production of bleached softwood and hardwood kraft pulps. Both effluents originated from the same mill. The bleaching sequence was in both cases O(C70 + D30)(EOP)D(EP)D. The chlorine multiple in the first bleaching stage was 0.09. HPSEC-conditions: TSK PW G3000 and G5000 columns connected in series. The mobile phase was 0.1 M Tris/HCl-buffer, pH 8.0. (Mörck et al. 1991)

in bleach liquors. Most of these studies have been directed toward the identification of chlorinated organic compounds. These studies were usually focused on liquors originating from bleaching of kraft pulps, using high charges of chlorine. So far, only a limited number of reports are available dealing with low-molecular weight organic compounds in effluent samples from chlorine dioxide, hydrogen peroxide, and ozone bleaching systems (Dahlman et al. 1995a; Strömberg et al. 1996). The analytical methods presented here are therefore mainly restricted to the results obtained in studies of liquors originating from chlorine bleaching. Although some of these methods are applicable to liquors from ECF bleaching, it should be kept in mind that the quantities of some low-molecular weight chlorinated compounds are orders of magnitude lower in ECF liquors than in liquors from chlorine bleaching, and that this may cause sensitivity and detection problems.

Other methods, originally described for liquors from chlorine bleaching, such as the determination of chlorinated phenolic compounds, have been extensively modified for ECF bleaching liquors using more sensitive and selective detection systems, especially highly sensitive gas chromatography/mass spectrometry (GC/MS) techniques.

Methods for determinations of different nonchlorinated compounds (such as fatty and resin acids) will also be discussed in the following. These methods are applicable to all types of bleaching liquors.

8.4.1 Carboxylic Acids

8.4.1.1 Hydrophilic Acids

Analysis of abundant organic hydrophilic acids in bleach liquors, such as formic, acetic, glycolic, and oxalic acids, can be performed rapidly without any prior derivatization, using ion chromatography (IC) with a suitable column and a gradient eluent. This is a useful method for determination of the most abundant carboxylic acids.

After derivatization, monocarboxylic acids (such as formic and acetic acids) can also be determined by gas chromatography (GC) as their benzyl esters (Alén and Sjöström 1991). Dicarboxylic acids and hydroxy acids have also been determined by GC after extraction and transformation to their trimethylsilyl derivatives (Alén and Sjöström 1991). Generally, GC provides better separation capacity than IC. In addition, GC combined with MS offers relevant information on the identity of the compounds studied.

Chlorinated acetic acids can easily be determined by GC equipped with an electron capture detector (ECD), after extraction and subsequent methylation using diazomethane. In bleach liquors from chlorine bleaching of kraft pulps, a number of chlorinated carboxylic acids have been determined using this

extraction and methylation method in combination with GC/MS (Lindström and Österberg 1986).

In the case of hydrophilic acids, no single analytical procedure can be recommended in preference to the other methods mentioned in this chapter. Consequently, for these compounds, the choice of analytical method must be dependent on the sample to be analyzed, the nature of the investigation, and the laboratory equipment available.

8.4.1.2 Lipophilic Acids

Because of their toxicity to fish and other water organisms, much attention has been paid to the analysis of the lipophilic fatty and resin acids. The fatty acids occurring in bleach liquors are mainly C_{16}–C_{24} straight-chain saturated and unsaturated aliphatic acids with an even number of carbon atoms. The resin acids are tricyclic diterpene carboxylic acids having two or more double bonds in the structure. Figure 8.2 shows examples of resin acids commonly found in bleach liquors from softwood kraft mills.

Common analytical procedures for the determination of individual fatty and resin acids in bleach liquors are based on extraction and derivatization followed by GC analysis using flame ionization detection (FID; Holmbom 1980). Their identification is usually based on GC/MS analysis. The choice of solvent and extraction conditions depends on the composition of the liquor sample to be analyzed. High concentrations of lipophilic extractives in the sample, which under certain conditions can form micelles or similar aggregates, can strongly influence the determination, by making the extraction of fatty and resin acids much less effective.

The concentrations of lipophilic extractives are usually much lower in liquors from kraft pulp bleaching than in liquors from sulfite pulp bleaching. Satisfactory results regarding the analysis of fatty and resin acids in liquors from kraft pulp bleaching can be obtained if the sample is first acidified and then subjected to the filtration and extraction procedure originally developed for steroids (see description under Sect. 8.4.3.6). After drying and evaporation of the solvent, the sample is methylated (diazomethane) and analyzed by GC/MS.

8.4.1.3 Chelating Agents

Chelating agents, such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), and nitrilotriacetic acid (NTA), are used to remove or deactivate trace metals in pulps before hydrogen peroxide bleaching. This is done in order to minimize hydrogen peroxide oxidation catalyzed by trace metals and subsequent decomposition of pulp polysaccharides.

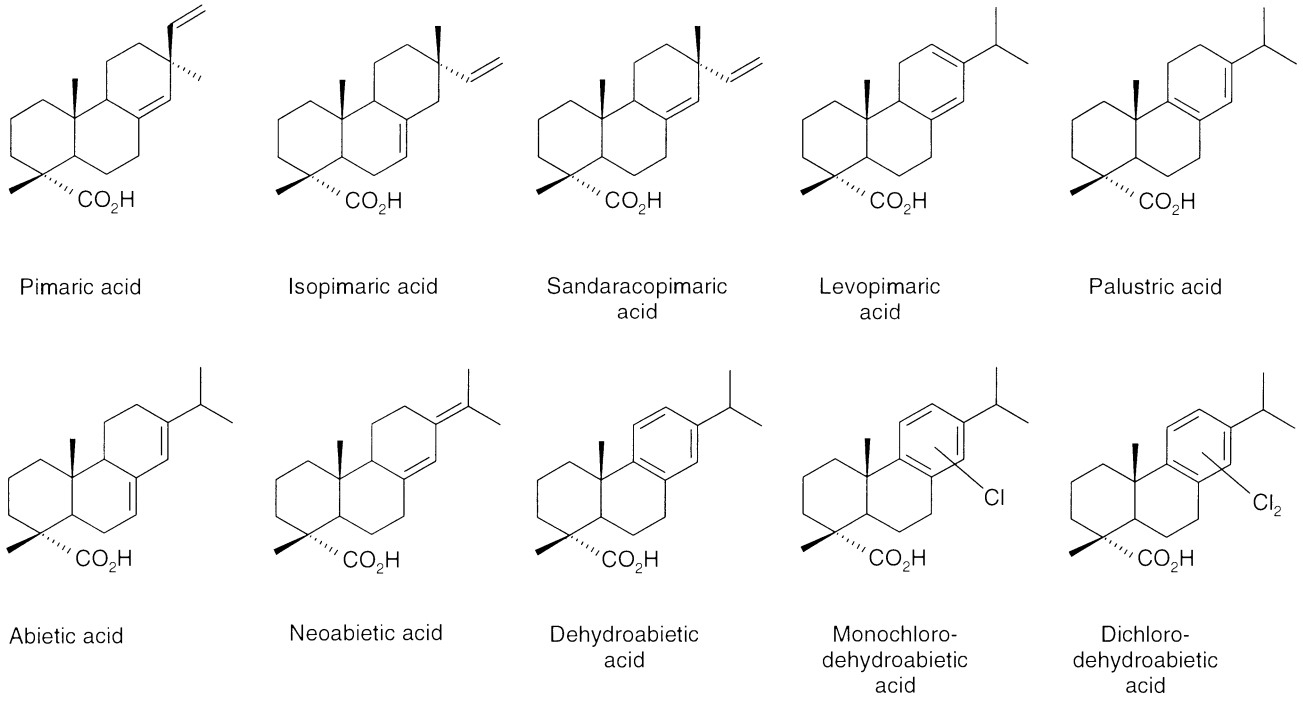

Fig. 8.2. Resin acids commonly found in effluents from production of softwood kraft pulp

Both GC (Nishikawa and Okumura 1995) and HPLC (Richardson et al. 1994; Sillanpää et al. 1995) techniques can be used for the determination of chelating agents. GC is, however, generally more sensitive and provides considerably better analytical separations than HPLC (Randt et al. 1993).

The determination of chelating agents in bleach liquors using capillary column GC with FID detection has proven to be both efficient and reliable. The liquor sample is first evaporated to dryness. The chelating agents in the sample are then converted to their methyl esters with boron trifluoride in methanol and finally analyzed by GC/FID. This method gives accurate determinations of EDTA and DTPA at concentrations lower than 0.1 mg/l. Figure 8.3 shows an example of a gas chromatogram of an effluent sample spiked with the chelating agents.

8.4.2 Phenolic Compounds

Phenolic compounds, especially highly chlorinated phenols, may have adverse effects on fish and other water organisms. The analysis of these compounds in bleach liquors has therefore attracted much attention.

In liquors from chlorine bleaching of pulp, tri- and tetrachlorinated phenolic compounds are often found in rather high concentrations, whereas the concentrations of these compounds in most cases are below the detection limit in liquors from mills with modern ECF bleaching. In the latter type of liquors, only minute amounts of mono- and dichlorinated phenolic compounds are usually detected.

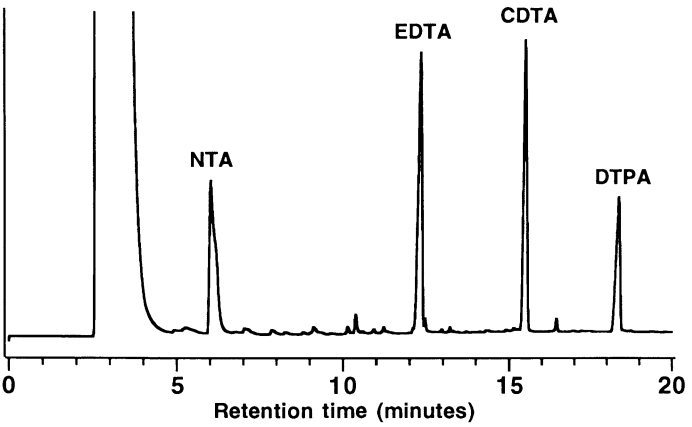

Fig. 8.3. Gas chromatographic separation of methylated chelating agents

Nonchlorinated phenolic compounds, which generally are less toxic to aquatic organisms than their chlorinated analogues, can be found in all types of bleach liquors.

8.4.2.1 Chlorinated Phenolic Compounds

Different procedures based on extraction of the liquor sample followed by derivatization and analysis of the extract by GC/ECD or GC/MS have been used for analysis of chlorinated phenolic compounds. Direct determination, without any derivatization, using HPLC has also been suggested.

The recommended method for the determination of chlorinated phenolic compounds in bleach liquors is, however, based on in situ acetylation using acetic anhydride, and is briefly described in the following. The liquor sample is first made alkaline by addition of potassium carbonate. Acetic anhydride is then added and the mixture is shaken vigorously to facilitate the acetylation reaction. The phenolic acetates obtained are extracted with *n*-hexane and analyzed by GC/ECD or GC/MS. This method is one of the most suitable and widely used routine procedures for the analysis of chlorinated phenolic compounds in bleach liquors. Thorough studies including interlaboratory comparisons have shown that most of the chlorinated phenolic compounds present in bleach liquors can be quantified down to concentrations of about 20 μg/l (Starck et al. 1985).

The availability of authentic reference compounds forms a basis for a reliable determination of chlorinated phenolic compounds in multicomponent mixtures such as those found in bleach liquors. The identification of individual chlorinated phenolic compounds in a sample must be based both on the comparison of GC retention times and mass spectra with those of the authentic reference compounds. As can be seen in Fig. 8.4, the mass spectra of 3,4,5- and 4,5,6-trichloroguaiacol are almost identical (Knuutinen and Korhonen 1984). Therefore, positive identification cannot be based only on mass spectral data. In such cases, GC retention times must also be used in order to make it possible to distinguish between the isomeric compounds.

Chlorinated phenols, catechols, and guaiacols are found in bleach liquors from bleaching of both softwood and hardwood kraft pulps, whereas compounds of the syringol type originate only from bleaching of hardwood kraft pulp. In Fig. 8.5, chemical structures are shown for some of the chlorinated phenolic compounds identified in bleach liquors. In liquors from chlorine bleaching of kraft pulps, catechols are dominant in the acidic C-stage liquors, whereas guaiacols dominate in the alkaline E-stage liquors.

As indicated previously, liquors from modern ECF bleaching of kraft pulps generally contain very small amounts of only a few chlorinated phenolic com-

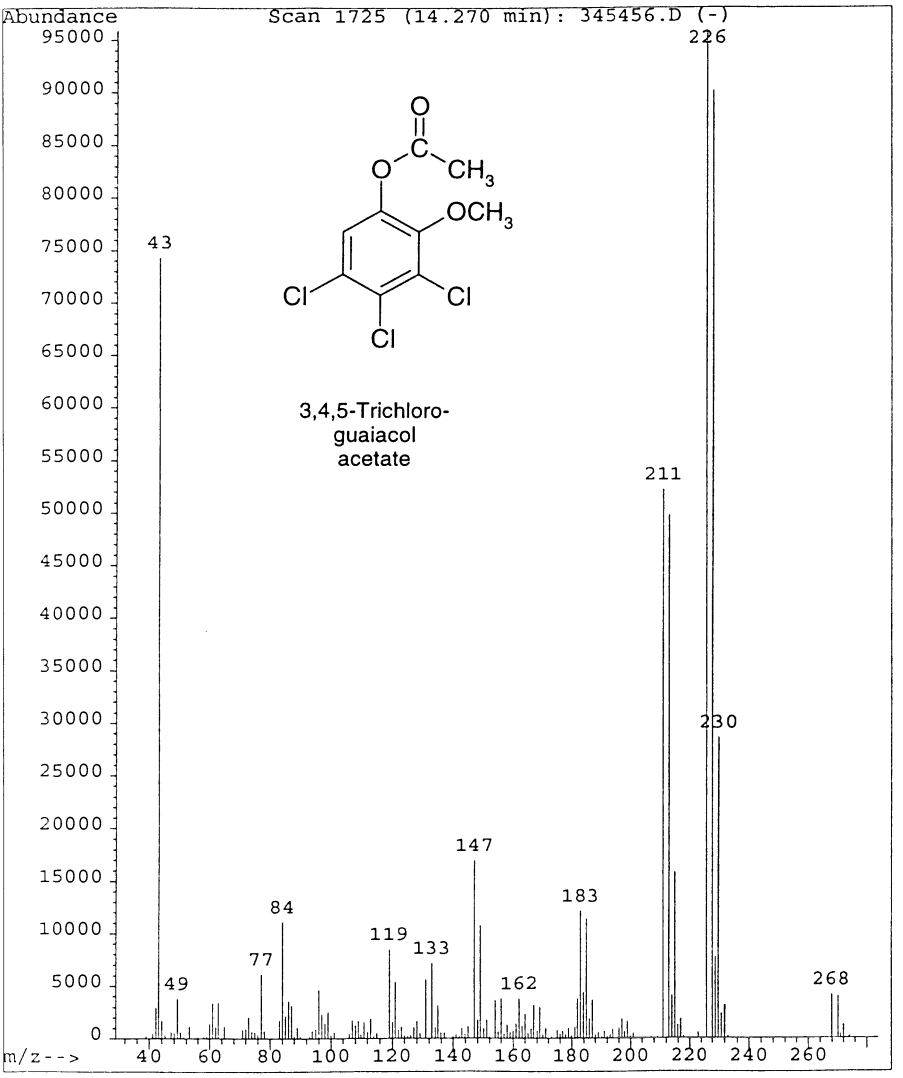

Fig. 8.4. Electron impact mass spectra of the acetyl derivatives of 3,4,5-trichloroguaiacol and 4,5,6-trichloroguaiacol. (Knuutinen and Korhonen 1984)

pounds. Since these compounds are mainly monochlorinated, GC/ECD is not a suitable analytical technique. This is due to the fact that ECD is a rather insensitive technique for detection of monochlorinated phenolic compounds. Other types of compounds, possibly nonchlorinated, which are present in higher concentrations, may therefore interfere with the determination and cause "false

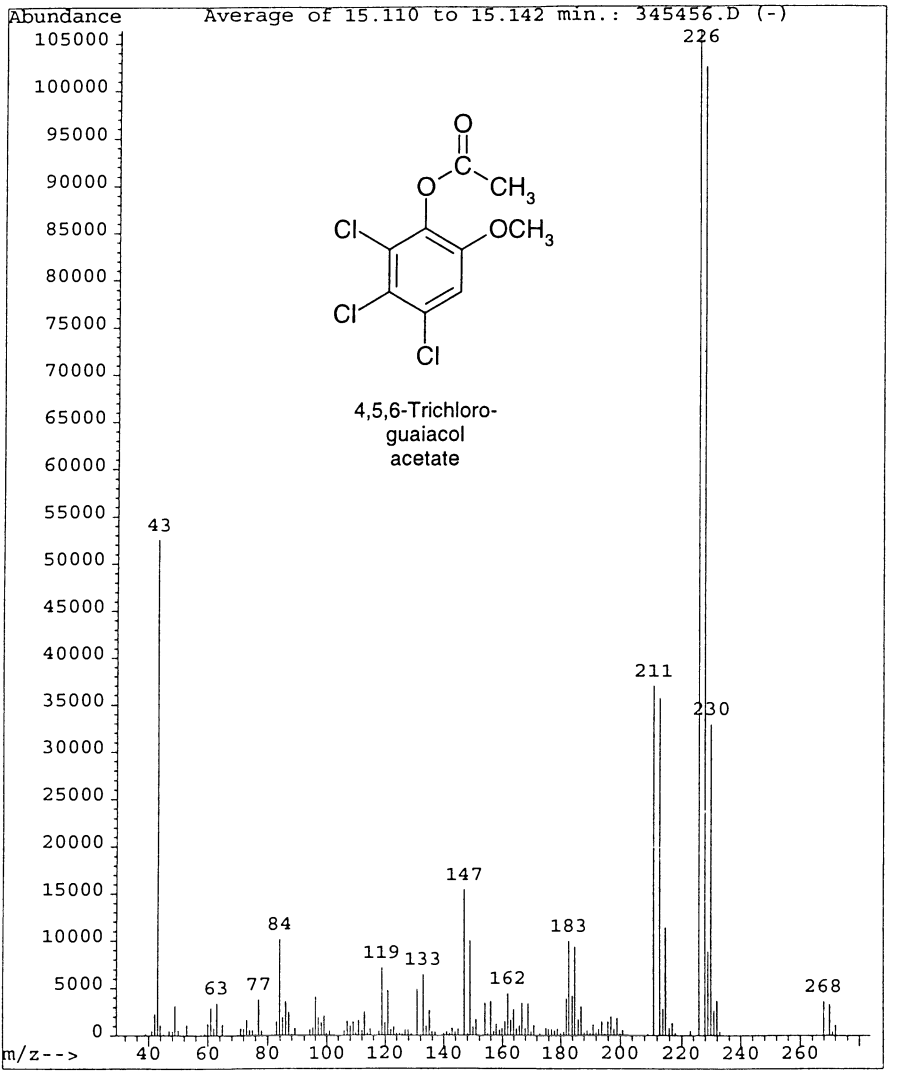

Fig. 8.4 *Continued*

positive" readings. GC/MS identification and quantification using appropriate isotopically labelled standard compounds (Deardorff et al. 1994) is therefore recommended for determination of the acetyl derivatives of chlorinated phenolic compounds in liquors from modern ECF bleaching.

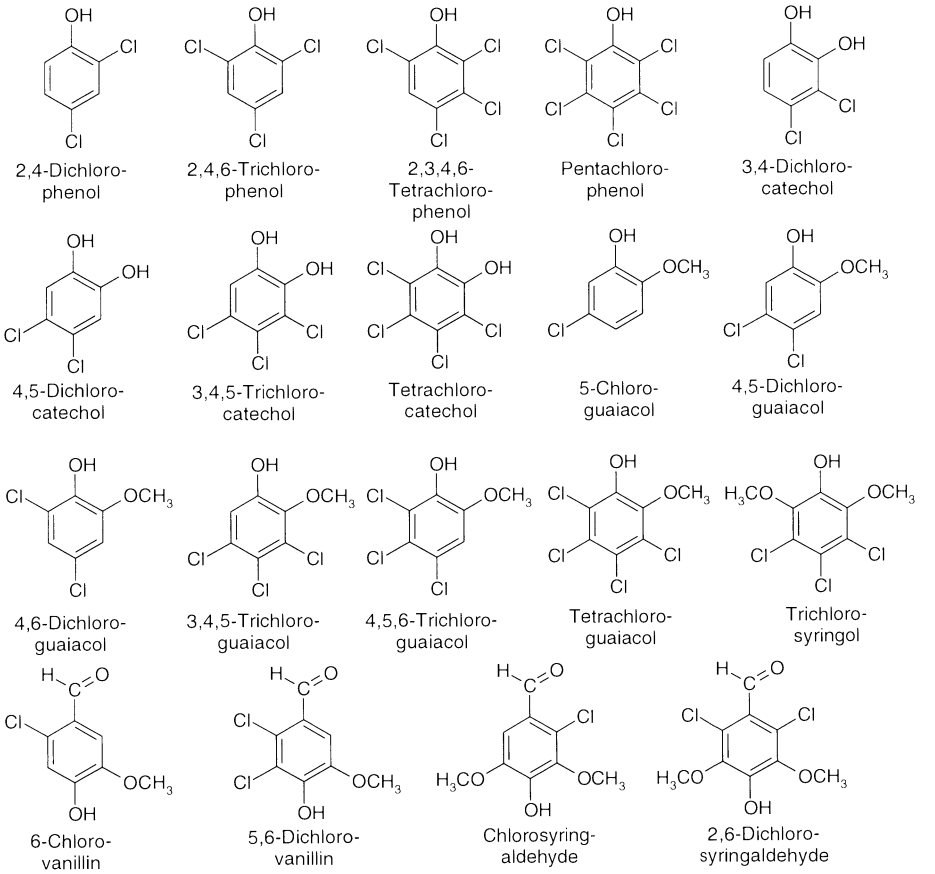

Fig. 8.5. Examples of chlorinated phenolic compounds identified in bleach liquors

8.4.2.2 Nonchlorinated Phenolic Compounds

For the determination of nonchlorinated phenolic compounds, an analytical technique based on extractive acylation using pentafluorobenzoyl (PFB) chloride has proven to be useful (Dahlman et al. 1995a). The resulting PFB derivatives can be separated and detected by GC/ECD. However, since other types of organic compounds, such as alcohols, also form PFB derivatives, GC/MS provides a more reliable identification and determination of the phenolic PFB derivatives. The liquor sample is first made alkaline (adjusted to pH 11) and is then washed once with *n*-hexane in order to remove neutral extractives. PFB chloride dissolved in *n*-hexane is thereafter added to the sample and the mixture is vigorously shaken. The hexane layer is then separated and worked up by usual techniques. The phenolic PFB derivates are finally analyzed by GC/ECD or GC/MS.

By using the extractive acylation techniques combined with GC/MS determination of PFB derivatives, phenolic compounds such as phenol, *o*-, *m*-, and *p*-methylphenol, guaiacol, 4-propenylphenol, 4-methylguaiacol, 4-ethylguaiacol, eugenol, vanillin, and acetoguaiacone have been found in liquors from production of softwood kraft pulp (Dahlman et al. 1995a).

8.4.3 Neutral Organic Compounds

The neutral fraction of the organic material in bleach liquors is composed of a large number of different types of organic compounds. In recent years, much attention has been paid to these types of compounds because the lipophilic character of some of them suggests a propensity to bioaccumulate in aquatic organisms.

Some of the neutral compounds in bleach liquors are naturally occurring wood extractives (e.g., steroids and triterpene alcohols), whereas other compounds (e.g., methanol, chloroform, and thiophenes) are degradation products of residual lignin or carbohydrates. The following discussion will be limited to those neutral compounds that, in recent literature, have been discussed in connection with environmental issues or with the chemistry of bleaching processes.

8.4.3.1 Methanol

Methanol can be found in both acidic and alkaline bleach liquors from chlorine or chlorine dioxide bleaching of kraft pulps (Pfister and Sjöström 1979). It is mainly generated from the methoxyl groups present in the residual pulp lignin.

Methanol can easily be determined by injecting the bleach liquor sample directly into a GC equipped with a suitable capillary column and FID detection. No other sample preparation than filtration for removal of suspended solids is needed (Ni et al. 1990).

8.4.3.2 Chloroform

Chloroform is formed both during chlorine and chlorine dioxide bleaching of kraft pulps. The largest amounts of chloroform were usually found in the alkaline hypochlorite stage liquors (Hrutfiord and Negri 1991). Liquors from ECF bleaching have been found to contain considerably smaller quantities of chloroform compared to liquors from conventional chlorine bleaching.

Two methods for the determination of chloroform have been described, both based on fairly simple analytical procedures using GC/ECD (Voss 1983)

or GC/MS (Lindström and Mohamed 1988). According to these methods, the sample is either extracted with *n*-pentane containing deuteriated chloroform as the internal standard (GC/MS method), or extracted with isooctane containing tetrachloroethylene as the internal standard (GC/ECD method). After drying, a small portion of the extract is injected directly into the GC.

Since chloroform is quite volatile, addition of the internal standard and extraction of the liquor should be carried out immediately after the liquor has been sampled.

8.4.3.3 Ketones and Aldehydes

In bleach liquors from chlorine bleaching of pulp, several chlorinated ketones (mainly chlorinated acetones) and aldehydes (e.g., 2-chloropropenal) have been identified. Some of the compounds identified have been found to exhibit mutagenic properties according to Ames test.

The quantitative determination of chlorinated acetones in bleach liquors is based on extraction with diethyl ether after acidification (pH 2) of the liquor followed by GC/MS (Kringstad et al. 1983) or GC/ECD analysis (McKague et al. 1990) of the extract obtained.

Derivatization with *O*-pentafluorobenzylhydroxylamine (PFBHA) hydrochloride followed by GC/MS analysis has been used for the characterization of carbonyl compounds in ozonated kraft pulps (Le Lacheur et al. 1993). Accordingly, low-molecular weight aldehydes and ketones present in bleach liquors can be determined as their *O*-pentafluorobenzyloximes (after aqueous-phase PFBHA-derivatization) using a procedure originally developed for analysis of ozonated drinking water. For identification and determination of the pentafluorobenzyloximes obtained, GC/MS is used. As an example, Fig. 8.6 shows a total ion current chromatogram corresponding to the analysis of PFBHA-derivatives of low-molecular weight aldehydes and ketones in a hardwood kraft bleach liquor (Dahlman et al. 1995a).

8.4.3.4 Dimethylsulfones

In bleach liquors from chlorine bleaching of kraft pulps, chlorinated dimethylsulfones are among the most abundant chlorinated low-molecular weight organosulfur compounds. These compounds are probably formed by oxidative chlorination of dimethylsulfide carried over to the bleach plant with black liquor residues. It has also been found that chlorinated dimethylsulfones (1,1-dichlorodimethylsulfone, 1,1,3-trichlorodimethylsulfone, and 1,1,3,3-tetrachlorodimethylsulfone) are rather resistant toward biological degradation. These types of compounds can therefore be found in relatively high

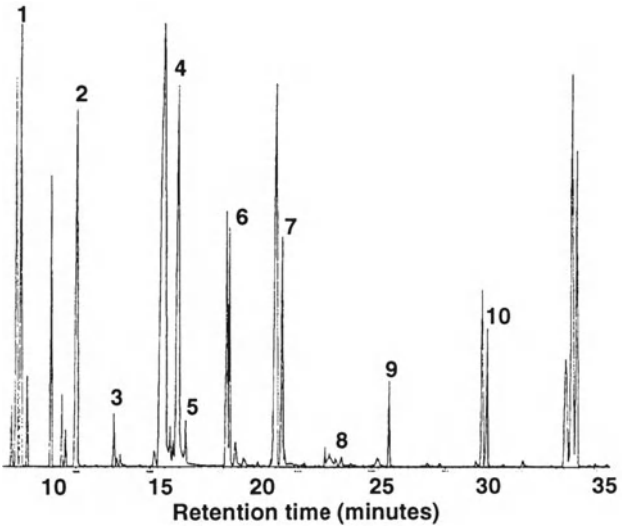

Fig. 8.6. Total ion current chromatogram of aldehydes and ketones (PFBHA derivatives) in ZP effluent from OQPZP bleaching of hardwood kraft pulp. Among the compounds identified were acetaldehyde (*1*), acetone (*2*), butanal (*3*), pentanal (*4*) 2-pentenal (*5*), hexanal (*6*), heptanal (*7*), nonanal (*9*), and glyoxal (*10*). (Dahlman et al. 1995a)

concentrations after biological treatment of whole mill effluents from production of chlorine-bleached pulps.

Determination of chlorinated dimethylsulfones in bleach liquors can be performed using a quite simple procedure based on extraction with diethyl ether, followed by GC/ECD analysis of the extract (Lindström and Mohamed 1988). Before extraction, the pH of the liquor should be adjusted to about 12.

8.4.3.5 Thiophenes

Thiophenes are formed by degradation of pulp carbohydrates during kraft cooking. A number of thiophenes with different substituents attached to the ring (mainly formyl and acetyl groups) has been identified in black liquors (Niemelä 1990). The thiophenes formed during kraft cooking can enter the bleach plant with the brownstock pulp and react with chlorine to form chlorinated thiophenes. Such compounds have been identified in bleach liquors from chlorine and chlorine dioxide bleaching of softwood and hardwood kraft pulps. The chemical structures of some chlorinated thiophenes identified in bleach liquors (both chlorination and alkaline extraction stage liquors) are shown in Fig. 8.7.

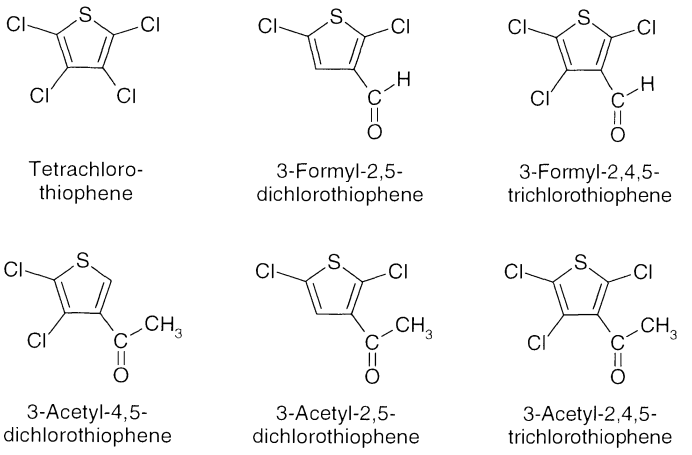

Fig. 8.7. Examples of chlorinated thiophenes identified in bleach liquors

The determination of chlorinated thiophenes in bleach liquors is based on extraction with cyclohexane or *n*-hexane (after adjustment of the pH of the liquor to about 2) followed by GC/MS analysis (Lunde et al. 1991) or GC/AED (atomic emission detection) analysis (Pedersen-Bjergaard et al. 1993) of the extract obtained.

8.4.3.6 Steroids and Triterpene Alcohols

The occurrence of wood-derived steroids in pulp mill effluents has recently attracted some attention, since it has been suggested that these steroids may cause physiological and biochemical responses in fish by acting similarly to hormones.

Steroids occur both in softwoods and hardwoods (see Chap. 5), often with sitosterol as the dominating component. The chemical structures of some steroids and triterpene alcohols commonly found in bleach liquors are shown in Fig. 8.8. Some of the triterpene alcohols shown in the figure (betulinol and lupeol) are hardwood (birch) constituents and are therefore found only in liquors from pulping of hardwood species (Holmbom 1980).

In bleach liquors, strongly lipophilic wood extractives such as steroids are, to a large extent, associated with suspended solids. Special attention must be paid to this behavior upon determination of these compounds. In order to obtain good extraction yields, a special extraction procedure has been developed (Dahlman et al. 1995a). According to this method, the liquor sample is first divided into suspended solids and water phase by filtration. The two fractions are then extracted separately with diethyl ether. The diethyl ether extracts obtained are combined and evaporated to dryness. After conversion to

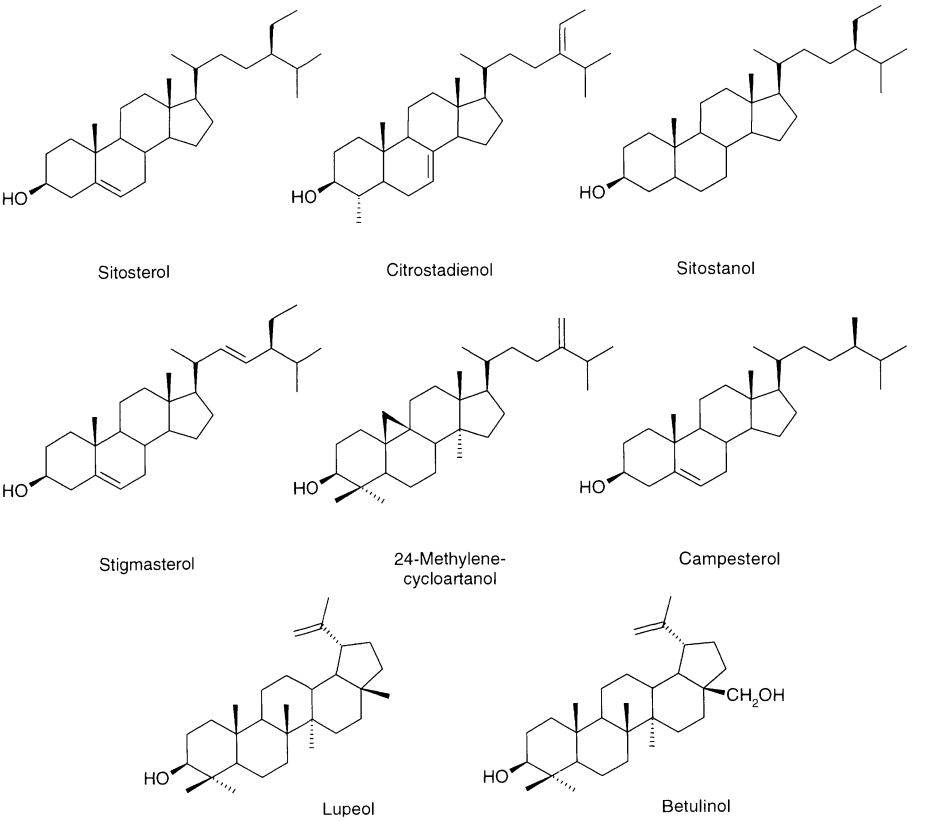

Fig. 8.8. Examples of steroids and triterpene alcohols identified in bleach liquors

trimethylsilyl ethers, the steroids and triterpene alcohols are determined by GC/FID or GC/MS.

8.4.3.7 Polychlorinated Dibenzo-*p*-Dioxins (PCDDs) and Dibenzofurans (PCDFs)

The discharge of PCDDs and PCDFs to water recipients from kraft pulp mills with chlorine bleaching attracted much attention from an environmental point of view during the late 1980s. The PCDDs and PCDFs found in liquors from chlorine bleaching are believed to be formed by chlorination of dibenzo-*p*-dioxin and dibenzofuran ("dioxin precursors") present at very low levels in the brownstock pulp entering the bleach plant.

The extremely small amounts of PCDDs and PCDFs present in bleach liquors from chlorine bleaching can only be detected by using highly sophisticated analytical techniques, including the use of isotopically labelled dioxins and expensive analytical equipment (i.e., highly sensitive high-resolution MS instruments). Determinations of PCDDs and PCDFs can thus only be performed at qualified analytical laboratories having very stringent quality assurance routines.

The basic principles for determinations of PCDDs and PCDFs in bleach liquors can be summarized as follows (Swanson et al. 1988). The bleach liquor sample is first divided into suspended solids and water phase by filtration. These fractions are then extracted separately. The two extracts obtained are combined, concentrated, and purified using a special clean-up procedure using three different clean-up columns (filled with silica gel/sulfuric acid, aluminum oxide, and activated carbon, respectively). The purified extract is analyzed by GC combined with a high-resolution MS operating in the selected ion monitoring (SIM) mode. The identification and quantification of the individual PCDD and PCDF congeners is carried out by comparison with isotopically labelled PCDDs and PCDFs added as standards to the sample before filtration and extraction.

8.4.4 Inorganics

Besides trace metals, only very few inorganic compounds in bleach liquors have been of interest from an environmental point of view. The following discussion will therefore be limited to the determination of chlorate (ClO_3^-) and chlorite (ClO_2^-) ions. The routine analysis of residual bleaching chemicals is not within the scope of this chapter.

IC with UV/Vis or conductivity detection is today a commonly used technique for the determination of inorganic anions in various sample solutions. In the case of liquors from chlorine dioxide bleaching stages, IC can be used for the determination of chlorate and chlorite (NCASI 1994). The bleach liquor is filtered (0.45 μm), injected on the IC column, and separated using an alkaline borate eluent gradient system. Both chlorate and chlorite ions can be detected by a conductivity detector. For more selective and sensitive detection of chlorite ions, a UV/Vis detector is, however, recommended.

8.5 Characterization of High-Molecular Weight Materials

The high-molecular weight material (HMWM) is a dominating part of the dissolved organic matter in bleach liquors. It is a heterogeneous material, mainly composed of strongly oxidized residual lignin fragments. The

HMWM may also contain oligo- and polysaccharides, mainly derived from hemicelluloses.

The HMWM is responsible for much of the COD and color discharged with bleach liquors. In effluents from bleaching with chlorine and chlorine-containing chemicals it also contains a significant part of the organically bound chlorine.

Some chemical characteristics of HMWMs resemble those of naturally occurring humic substances. HMWMs are, like these substances, rich in carboxyl groups and may under neutral or alkaline conditions behave as anionic polyelectrolytes, which can attract and carry substantial amounts of metal ions. It has also been suggested that HMWMs, like humic substances, can act as a macromolecular carrier for smaller, highly lipophilic organic compounds, thereby affecting their transport in the aquatic environment, while at the same time reducing their bioavailability.

With the techniques currently available, only a partial enrichment and characterization of individual high-molecular weight lignin degradation products is possible. A practicable way is, therefore, to characterize such materials with respect to sum parameters such as molecular weight distribution, elemental composition, and contents of functional groups. More detailed structural information may be obtained by applying different chemical degradation procedures and spectroscopic methods such as ^{13}C NMR on isolated HMWMs.

Methods described elsewhere in this book for determination of functional groups in lignin (Chap. 4) can also be applied to freeze-dried HMWMs and are therefore not discussed here.

8.5.1 Isolation

Ultrafiltration (UF) has been frequently used for isolation of preparative amounts of high-molecular weight material from bleach liquors, and is probably the best and most convenient method today available for this purpose. Other procedures, such as acid precipitation and XAD adsorption, are less suitable, since they are too unspecific with respect to molecular weight.

In many investigations, UF membranes with nominal cutoff at 1000 Da have been used mainly because organic molecules with molecular weights >1000 Da are believed to be biologically inactive.

It should be kept in mind that the UF separation is related not only to molecular weight. It also depends on the shape of the molecules, which may cause a certain overlap between the permeate and concentrate fractions. This is illustrated in Fig. 8.9, which shows the results of ultrafiltration (nominal cutoff 1000 Da) of an industrial bleach plant effluent from ECF bleaching of softwood kraft pulp (Mörck et al. unpubl. results).

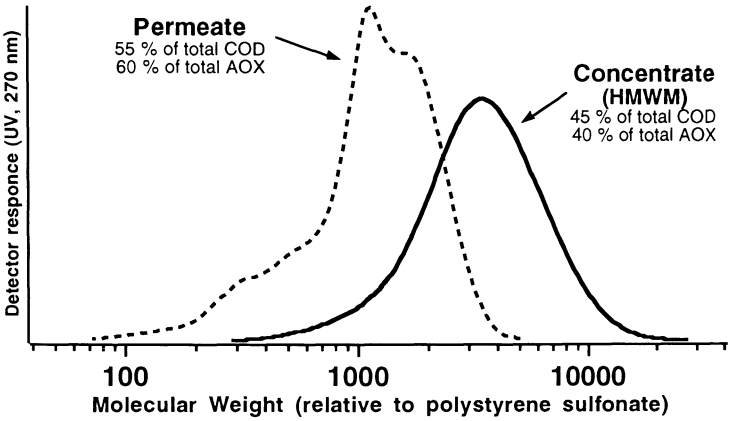

Fig. 8.9. Apparent molecular weight distributions (HPSEC) of the UV-absorbing organic materials in the permeate and concentrate obtained upon ultrafiltration (nominal cutoff 1000 Da) and subsequent diafiltration of an industrial bleach plant effluent from production of ECF bleached softwood kraft pulp. The bleaching sequence was OD(EOP)DED. (R. Mörck et al., unpubl. results)

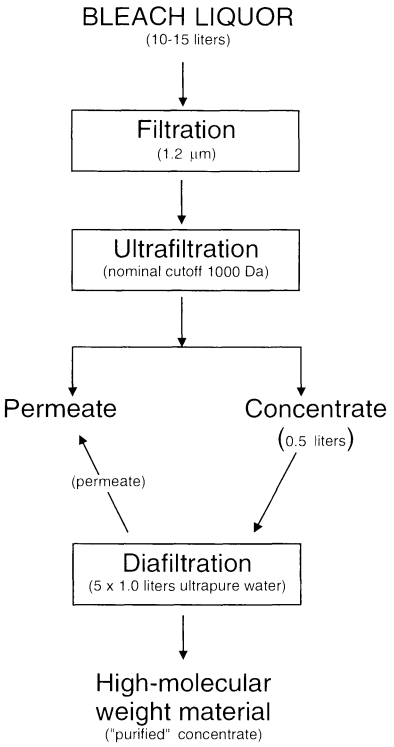

Fig. 8.10. Ultrafiltration procedure for isolation of HMWMs from bleach liquors

Figure 8.10 schematically illustrates a commonly used ultrafiltration procedure for isolation of the HMWMs from bleach liquors (Dahlman et al. 1995b). Before ultrafiltration, the effluent should be prefiltered in order to remove suspended solids. This is important in order to make sure that the UF concentrate contains only dissolved material, and to avoid clogging of the UF membrane. The use of tangential flow UF equipment is recommended. In such equipment, the bleach liquor flows across the membrane, which keeps the membrane surface clean and facilitates the penetration of small molecules through the membrane pores. It is also important to make sure that the UF equipment used is capable of concentrating the liquor 10–20 times. The UF membranes should preferably be low-adsorbing polysulfone.

After ultrafiltration, the concentrate (which contains the HMWMs) is subjected to a washing procedure (sequential batch diafiltration) in order to remove residues of low-molecular weight organic compounds and inorganic salts. The concentrate is first diluted with pure water and again subjected to ultrafiltration. This washing procedure should be repeated five times. The final concentrate after diafiltration is normally freeze-dried in order to obtain a dry HMWM suitable for various types of analyses.

8.5.2 Chemical Degradation Methods

Chemical methods used for studies on the structure of lignins or lignin-derived materials often involve degradation of the materials. Such methods therefore produce indirect structural information and may also suffer from the drawback that both the investigated material and the degradation products obtained may have undergone structural changes other than those intended to happen. However, chemical degradation of HMWMs by permanganate oxidation, cupric oxide oxidation or thioacidolysis provides valuable information concerning the composition and the degree of chlorination of the residual aromatic structural elements in the lignin-derived parts of HMWMs. In these methods, the HMWMs are degraded into low-molecular weight compounds that are amenable to analysis by using GC, HPLC, and GC/MS. The method does not, however, permit a quantitative determination of the total number of aromatic structures in the sample.

8.5.2.1 Permanganate Oxidation

The permanganate oxidation method used in recent investigations on HMWMs from kraft mill effluents (Mörck et al. 1991; Dahlman et al. 1993, 1995b) is essentially the same as the lignin degradation method described in Chapter 4. This section will therefore only discuss the degradation products obtained by permanganate oxidation of HMWMs.

The major types of lignin-related aromatic degradation products normally obtained by permanganate oxidation of bleach liquor HMWMs are shown in Fig. 8.11. The lignin degradation products A–E originate from residual 4-hydroxyphenyl (A), 3,4-dihydroxyphenyl (B), guaiacyl (C), "condensed"

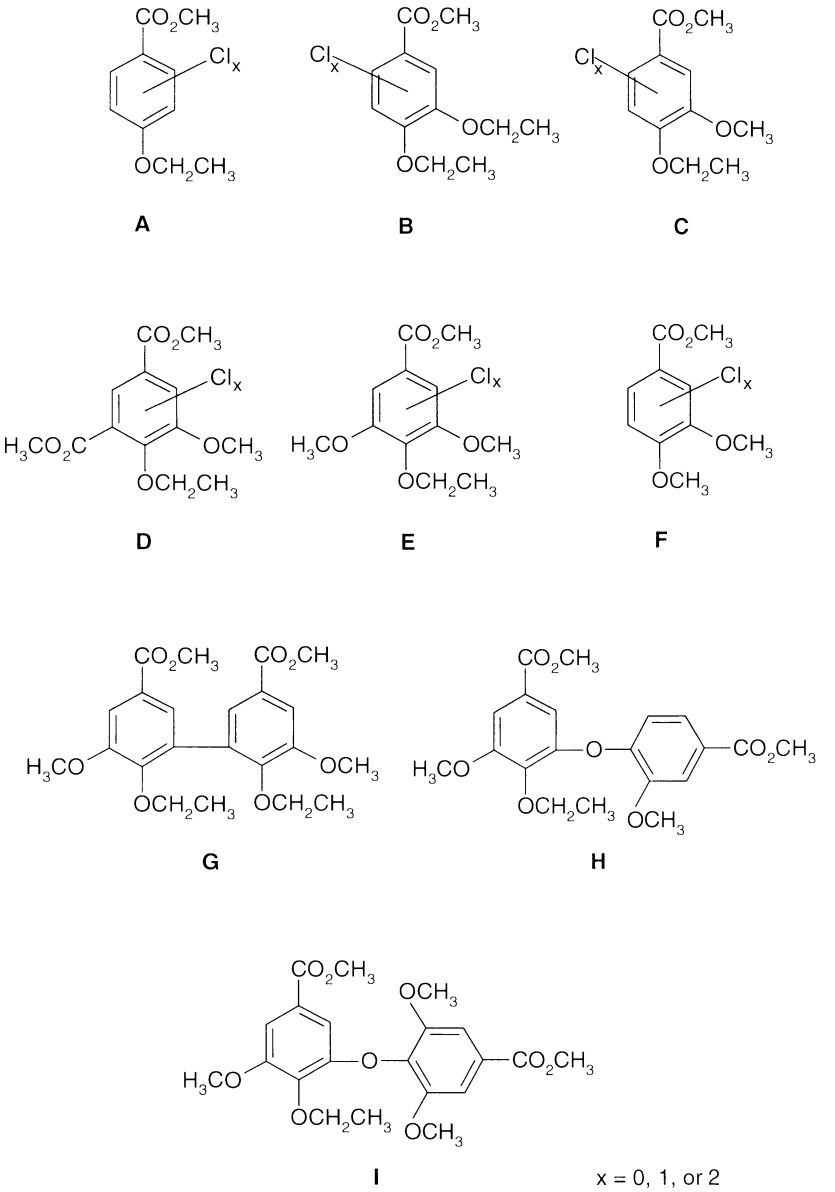

Fig. 8.11. Common types of lignin-related aromatic degradation products obtained upon permanganate oxidation of HMWMs. (Dahlman et al. 1995b)

guaiacyl (D), and syringyl (E) units present in bleach liquor HMWMs. Degradation products of the veratryl type (F) are sometimes also found. It is, however, still not known whether the veratryl degradation products originate from the residual pulp lignin. In addition to the monomeric aromatic degradation products obtained (A–F), three lignin-derived dimeric degradation products (G–I) originating from diphenyl (G) and diphenyl ether units (H, I) have been reported (Dahlman et al. 1995b).

Permanganate oxidation of chlorine bleach effluent HMWMs, which originate from kraft pulp mill, yields, in addition to the nonchlorinated degradation products, monochlorinated and dichlorinated degradation products of the types A–F. Trichlorinated degradation products are seldom observed.

Figure 8.12 shows as an example the total ion current chromatogram of the monomeric degradation products obtained upon permanganate oxidation of a whole mill effluent HMWM originating from a softwood kraft mill with chlorine bleaching (O. Dahlman et al., unpubl. results). As expected, degradation

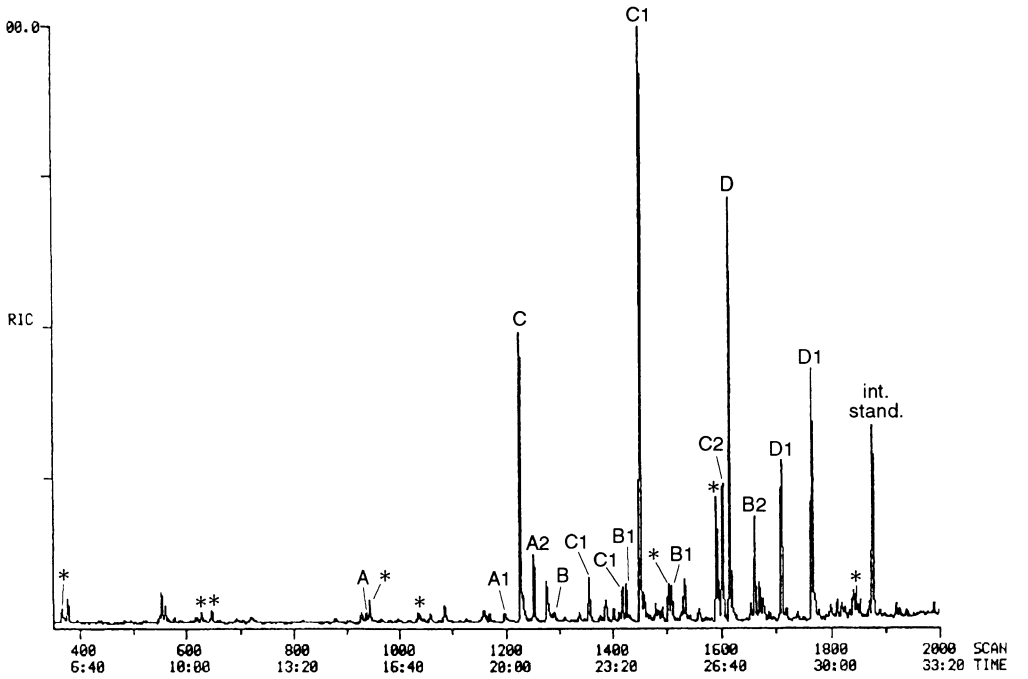

Fig. 8.12. Total ion current chromatogram of monomeric degradation products obtained upon permanganate oxidation of a HMWM sample isolated from a softwood kraft whole mill effluent. The bleaching sequence used was O(C70 + D30)(EOP)D(EP)D. The numbers in the figure denote the number of chlorine atoms in chlorinated aromatic degradation products of types *A–D* (for structures see Fig. 8.11). *Asterisks* (*) denote aromatic products not related to lignin (mainly mono, di, and tricarboxylated benzenes). (O. Dahlman et al,. unpubl. results)

products originating from structural elements of the guaiacyl (C) and "condensed" guaiacyl (D) types constitute the dominating peaks in the chromatogram. Several monochlorinated degradation products (types A–D, labeled A1, B1, C1 and D1) were observed. All three possible isomers of monochlorinated C were identified, as well as the two possible isomers of monochlorinated D. The largest peak in the chromatogram in Fig. 8.12 originated from one of the monochlorinated C-isomers (chlorine atom in position 6). The mass spectrum of this compound is shown in Fig. 8.13. Peaks originating from dichlorinated degradation products (types A, B, and C, labeled A2, B2, and C2) were also found in the chromatogram.

The quantities of chlorinated degradation products (mainly monochlorinated) obtained upon permanganate oxidation of HMWMs from kraft mills with ECF bleaching have been found to be very small. This is especially true for HMWMs dissolved during ECF bleaching of pulps from modified kraft cooking processes involving oxygen delignification to low kappa numbers (Dahlman et al. 1993, 1995b).

Chlorinated dimeric degradation products of types G–I have to the best of our knowledge not been reported upon permanganate oxidation of HMWMs.

It should be pointed out that permanganate oxidation of HMWMs from oxidative bleaching processes often requires larger amounts of sample than studies on nonoxidized lignins, since HMWMs from oxidative bleaching processes normally exhibit a lower content of phenolic structural elements than nonoxidized lignins.

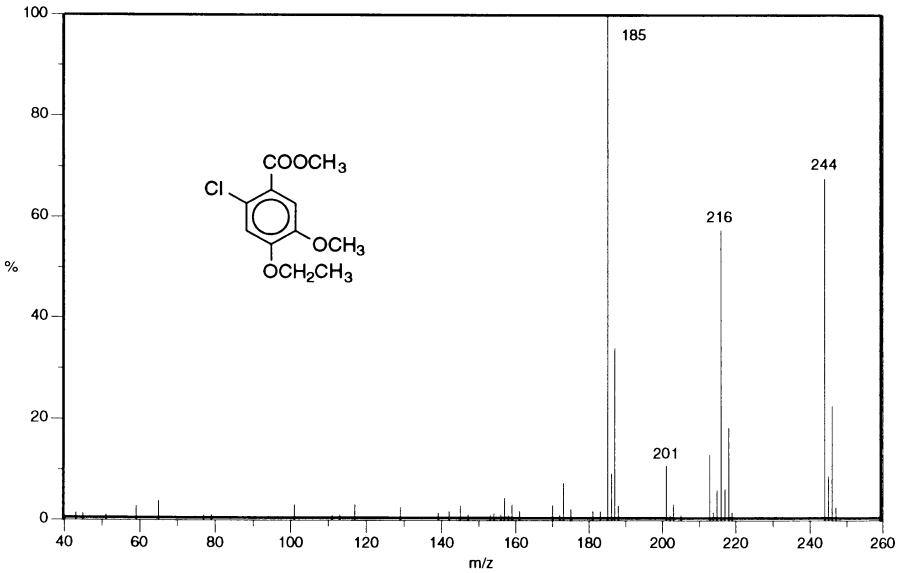

Fig. 8.13. Mass spectrum of the methyl ester of 6-chloro-4-ethoxy-3-methoxybenzoic acid (dominating compound of *C1* in Fig. 8.12). (O. Dahlman et al., unpubl. results)

8.5.2.2 Cupric Oxide Oxidation

Cupric oxide (CuO) oxidation has also been used to characterize lignin-derived aromatic structural elements in HMWMs. Like permanganate oxidation, CuO oxidation gives information on the composition of the residual aromatic structural elements in HMWMs. The reaction mechanism for CuO oxidation of lignins and lignin-derived materials is, however, not well established, although CuO is known to be a one-electron transfer oxidant (Chen 1992).

The major monomeric aromatic degradation products obtained on CuO oxidation of HMWMs are phenolic aldehydes. Smaller amounts of phenolic acids and ketones have also been observed. Like permanganate oxidation, the method does not permit a quantitative determination of the total content of aromatic structures in HMWMs.

The recommended procedure for CuO oxidation of HMWMs (Hyötyläinen et al. 1995) is illustrated in Fig. 8.14. A mixture of HMWM, cupric oxide, and iron ammonium sulfate hexahydrate in a 2 M sodium hydroxide solution is heated in a teflon-coated bomb at 170 °C for 2 h and is thereafter allowed to cool to room temperature. After oxidation, the reaction mixture, which contains phenolic degradation products, is centrifuged to separate the inorganics. The precipitate is washed with water and the combined aqueous phases are acidified with 6 M hydrochloric acid (to pH 3). After standing for 1 h, the cleanup is performed using a C-18 microcolumn. The identification and quantification of the degradation products can be carried out by reversed phase HPLC with photodiode array detection, where the retention times of the peaks are compared with those of authentic model compounds. After silylation of the degradation products, identification, and quantification of the degradation products can also be carried out by GC/MS.

Normally, only monomeric degradation products originating from uncondensed structures are obtained on CuO oxidation of HMWMs. In addition to the monomeric products, two dimeric aromatic degradation products originating from biphenyl structures have been identified (Hyötyläinen et al. 1995). The main degradation products formed upon CuO oxidation of chlorinated HMWMs are presented in Fig. 8.15.

An example of a reversed phase HPLC chromatogram of the degradation products is shown in Fig. 8.16. As can be seen, the dominating degradation product in the chromatogram is vanillin, but also several other aldehydes, phenolic acids, and ketones (including some chlorinated ones) have also been identified by comparison with retention times of model compounds.

The most abundant chlorinated degradation product in the chromatogram in Fig. 8.16 is 6-chlorovanillin. This agrees with results from permanganate oxidation of chlorinated HMWMs and indicates that the 6-chloro isomer is the dominating isomer among the monochlorinated guaiacyl structural elements in chlorinated HMWMs.

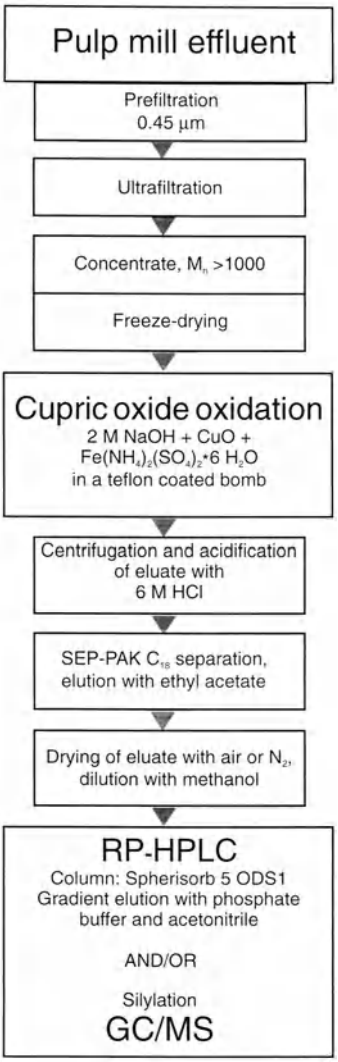

Fig. 8.14. The main principles of the cupric oxide oxidation method

8.5.2.3 Thioacidolysis

Chemical degradation based on thioacidolysis according to the method developed by Rolando et al. 1992, has been used to gain information of alkyl-aryl ether structures in native and industrial lignins. The method can also be used to assess whether or not residual β-*O*-4-structures occur in HMWMs from various types of bleach liquors.

The thioacidolysis reaction, which proceeds via acidic cleavage of α-*O*-4 and β-*O*-4 linkages, results in depolymerization of the lignin sample with

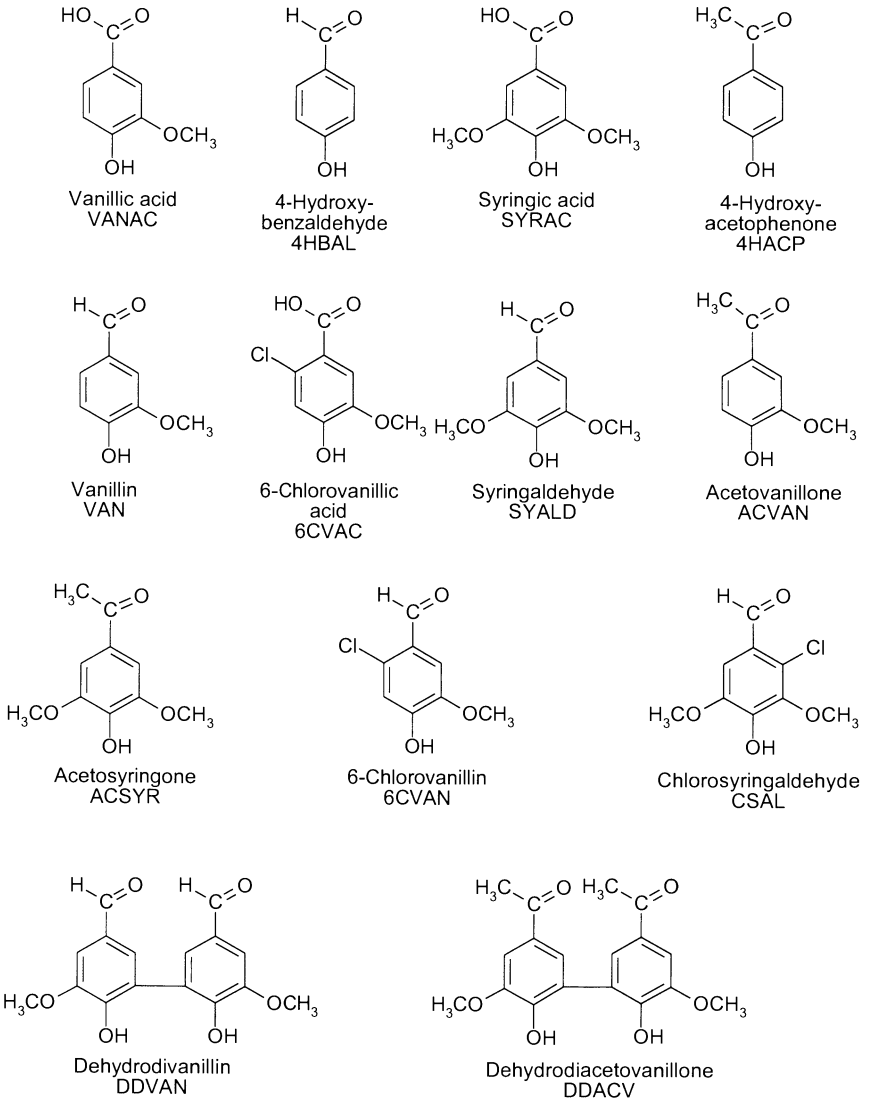

Fig. 8.15. Structures and names of the main degradation products formed during cupric oxide oxidation of bleach liquor HMWMs

formation of trithioethylphenylpropane compounds. Thus, low-molecular weight lignin-derived degradation products are formed, which are amenable to analysis by GC/MS.

Upon thioacidolysis of HMWMs from ECF and TCF bleaching processes, small quantities (0.1–0.3% of dry starting material) of thioacidolysis degrada-

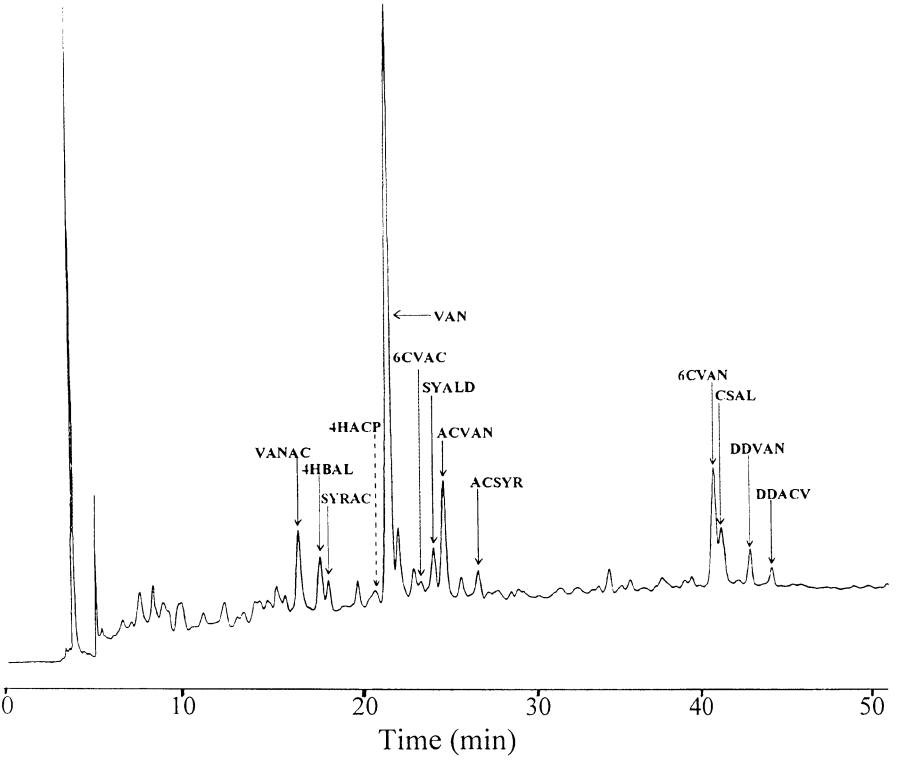

Fig. 8.16. Reversed phase HPLC trace of degradation products formed upon CuO oxidation of a HMWM sample isolated from a whole kraft pulp mill effluent. For names, see Fig. 8.15. (Hyötyläinen et al. 1995)

tion products originating from guaiacyl and syringyl structural elements have been detected (Dahlman et al. 1995b). In addition, trace amounts of monochlorinated thioacidolysis products (both guaiacyl and syringyl types) have been detected by GC/MS analysis in the selected ion monitoring (SIM) mode after thioacidolysis of the HMWM from an ECF kraft bleach plant effluent (Dahlman et al. 1996b).

A recommended procedure for thioacidolysis of HMWMs is as follows. The freeze-dried HMWM sample is kept overnight in a desiccator over P_2O_5 and is then suspended in a mixture (9:1) of dried dioxane and ethanethiol containing 0.2 M BF_3-etherate. The reaction mixture is deaerated (argon), heated at 100 °C for 4 h, cooled to room temperature, poured into a 0.4 M sodium carbonate solution, and extracted with dichloromethane. The dichloromethane extract is dried (sodium sulfate), filtered, evaporated to a small volume, and silylated using *N*, *O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The

degradation products can now be analyzed as their trimethylsilyl ethers by GC/MS.

8.5.3 Carbohydrate Content and Composition

Almost all carbohydrates present in bleach liquors are found in the UF concentrates, and they are thus oligo- and polysaccharide constituents of the HMWMs (Pfister and Sjöström 1979). Only traces of carbohydrates are usually present in the UF permeates. The amount and composition of carbohydrates in bleach liquors can therefore be determined by carbohydrate analysis of freeze-dried HMWMs.

The dominating part of the comparatively small quantities of carbohydrates (6–9% of the HMWM) found in bleach liquors from chlorine (Pfister and Sjöström 1979) or chlorine dioxide bleaching (Dahlman et al. 1995b) of softwood kraft pulps are dissolved in the alkaline extraction stage. Somewhat larger quantities of carbohydrates (13–18% of the HMWM) can be found in bleach liquors from chlorine dioxide bleaching of hardwood kraft pulps (Dahlman et al. 1995b).

Hydrogen peroxide-stage bleach liquors from TCF bleaching of softwood and hardwood kraft pulps contain larger quantities of carbohydrates (about 14% and 40%, respectively) than the corresponding alkaline extraction liquors from ECF bleaching (Dahlman et al. 1995b). The extraction of hemicelluloses from the kraft pulp thus seems to be favored by the conditions used in a normal or pressurized hydrogen peroxide bleaching stage. In contrast, only small amounts for carbohydrates have been found in ozone-stage liquors (Dahlman et al. 1996a).

All types of bleach liquor HMWMs, especially hardwood-derived HMWMs, contain carbohydrates rich in xylose (Dahlman et al. 1995b). Thus, hemicelluloses of the xylan type are the dominating carbohydrate constituent in bleach liquor HMWMs (Pfister and Sjöström 1979; Dahlman et al. 1995b). Carbohydrate component analyses also indicate that softwood-derived bleach liquor HMWMs contain some galactoglucomannan. However, the composition of the bleach liquor galactoglucomannan (low mannose content) seems to differ considerably from that of native galactoglucomannan in wood.

In order to determine the carbohydrate composition of bleach liquor HMWMs, the oligo- and polysaccharides must be hydrolyzed to monosaccharides that are amenable to subsequent analysis by GC (TAPPI 1985), IC (Laver and Wilson 1993) or capillary electrophoresis (Rydlund and Dahlman 1996; Dahlman et al. 1997). There are a number of acid hydrolysis methods by which oligo- and polysaccharides can be converted into their monosaccharide moieties. One of these, used by the present authors, is that suggested by Theander and Westerlund 1986. The acid hydrolysis method is described elsewhere in this book (Chap. 3).

8.5.4 ^{13}C NMR Spectroscopy

Spectroscopic methods for structural analysis of HMWMs isolated from bleach liquors are very important and valuable complements to the chemical degradation methods. ^{13}C NMR spectroscopy is one of the most powerful spectroscopic techniques available today for structural studies on organic materials (see Chaps. 3 and 4). This section will discuss various aspects of ^{13}C NMR spectroscopy of bleach liquor HMWMs.

Solution-state ^{13}C NMR spectra of HMWMs from bleach liquors usually consist of a number of broad and rather poorly resolved signals. Signals originating from carbohydrate structures are, however, generally sharper than those derived from lignin structures. The poor resolution of the signals from lignin-derived structures is probably due to a combination of several factors such as the comparatively high molecular weight, the heterogeneous chemical structure, and the presence of small amounts of complexes with paramagnetic transition elements, which may all cause line broadening. It is, however, important to point out that solution-state ^{13}C NMR spectra of HMWMs provide valuable structural information concerning both the lignin-derived part of the material and the carbohydrate part, in spite of the rather broad signals.

In solid-state ^{13}C NMR spectra of HMWMs, the line broadening is much more pronounced than in solution-state ^{13}C NMR spectra, which severely limits the value of the solid-state technique in the characterization of HMWMs from bleach liquors. This is exemplified in Fig. 8.17, which shows both the solid-state and the solution-state (solvent: DMSO-d_6) ^{13}C NMR spectra of a softwood-derived HMWM sample from an ECF kraft bleach plant liquor (R. Mörck et al. unpubl. results).

Solution-state ^{13}C NMR spectra of HMWMs can be recorded either from water (D_2O) solution or from solutions in deuteriated organic solvents. A suitable HMWM concentration is 100–300 mg/ml (10 mm tube is recommended). It is, however, often difficult to obtain such concentrated solutions of HMWMs in D_2O. Hexadeuteriated dimethyl sulfoxide (DMSO-d_6) is therefore recommended as the solvent in ^{13}C NMR studies on HMWMs (Dahlman et al. 1995b; Mörck et al. 1995). After treatment with a strongly acidic ion exchanger (such as Amberlite IR-120 or Dowex 50 W-X8) followed by freeze-drying, it is normally easy to prepare concentrated solutions of HMWMs in DMSO-d_6. The solutions may, however, be somewhat viscous at room temperature. In such cases, the spectra can be recorded at 50–80 °C in order to lower the solvent viscosity and to improve the quality of the spectra obtained.

Solution-state spectra of HMWMs from oxidative bleaching processes normally show two broad and intense signals in the carbonyl region assigned to carboxylic acid carbons (Dahlman et al. 1995b; Mörck et al. 1995). The chemical shifts of these signals (signals 1 and 2 in Fig. 8.17) indicate that signal 1 mainly originates from carbon atoms in carboxylic acid groups bound to saturated aliphatic structures (Kringstad and Mörck 1983), whereas signal 2 may

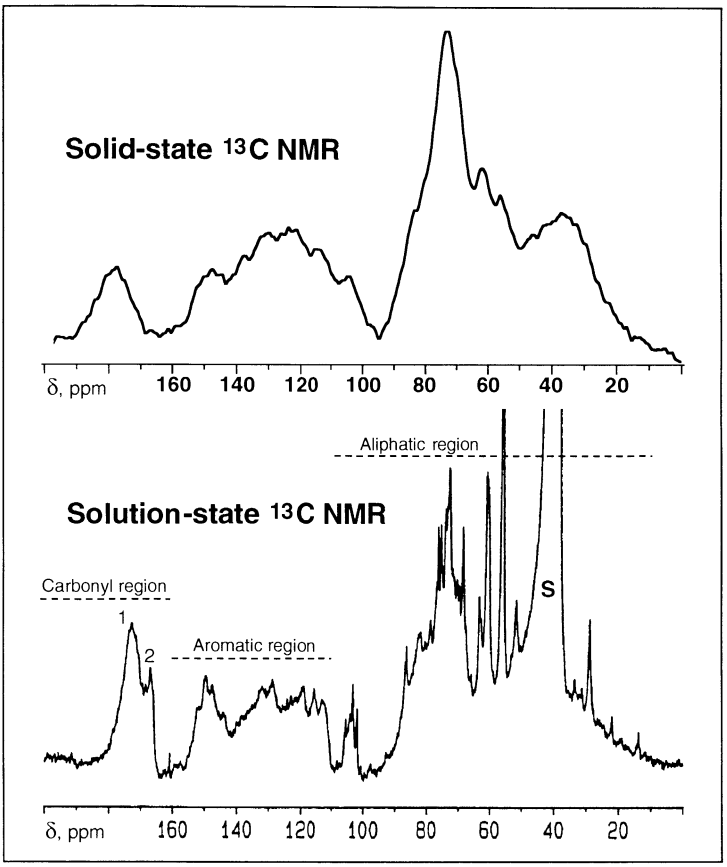

Fig. 8.17. Solid-state and solution-state ^{13}C NMR spectra of an industrial bleach plant effluent HMWM from production of ECF-bleached softwood kraft pulp. The bleaching sequence was OD(EOP)DED. *S* solvent (DMSO-d_6). (R. Mörck et al., unpubl. results)

represent carbon atoms in carboxylic acid groups conjugated to aromatic or olefinic structures (Kringstad and Mörck 1983).

The signal pattern in the aromatic region (Fig. 8.17) gives information about whether the HMWM sample studied contains significant quantities of lignin-related aromatic structural elements or not. Recent ^{13}C NMR studies (Dahlman et al. 1995b; Mörck et al. 1991, 1995) on HMWMs from various bleaching processes have shown that hardwood-derived HMWMs from kraft bleaching processes based on chlorine, chlorine dioxide, hydrogen peroxide or hydrogen peroxide/ozone are mainly nonaromatic, whereas aromatic lignin structures are retained to a significant degree in softwood-derived HMWMs from chlorine dioxide or hydrogen peroxide bleaching.

^{13}C and ^{1}H NMR spectra of HMWM samples have revealed the presence of unsaturated uronic acid units (4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic

acid units) in the xylan dissolved from kraft pulp during hydrogen peroxide bleaching (Dahlman et al. 1996a; Mörck et al. 1995). Characteristic signals from some of the carbons in these units (C-4, C-5, and C-6) are found in the aromatic and carbonyl regions in ^{13}C NMR spectra of hardwood-derived HMWMs. These signals are, however, observed less frequently in spectra of softwood-derived hydrogen peroxide stage liquor HMWMs due to the lower content of xylan in such samples. The unsaturated uronic acid units give characteristics signals (H-4 and H-1) also in ^{1}H NMR spectra and can be quantified (both in softwood and hardwood-derived samples) in relation to xylose units by use of ^{1}H NMR spectroscopy (Dahlman et al. 1996a).

The aliphatic region in solution-state ^{13}C NMR spectra of HMWMs (Fig. 8.17) shows signals from carbohydrate carbons and carbons in aliphatic side-chains in residual lignin structures.

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CPPA 1991 Determination of biochemical oxygen demand. Canadian Pulp and Paper Association. Technical Section, Montreal, Standard H.2

CPPA 1993 Chemical oxygen demand. Canadian Pulp and Paper Association. Technical Section, Montreal, Standard H.3

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9 Analysis of Papermaking Process Waters and Effluents

B. Holmbom

9.1 Introduction

Not only pulping, but also papermaking, involves complex chemical phenomena. Dissolved and colloidal substances are released into process waters from pulps and are carried over to papermaking operations. This material represents a multitude of different components, which interact with one another and with the pulp fibers, fillers, and process chemicals at the wet end of the paper machine. Along with the trend of minimizing the use of fresh water until the water system is nearly a closed cycle, the concentrations of dissolved and colloidal substances are increasing in papermaking waters. The highly turbulent conditions, the varying pH, and the presence of microorganisms are further factors to be taken into account when managing the wet-end chemistry. This is of critical importance, both for paper machine runnability and paper quality. Furthermore, these dissolved and colloidal substances will end up in the mill effluents, if not carried with the paper or taken out in the form of solid rejects.

A significant amount of wood material is dissolved and dispersed in mechanical pulping and the subsequent bleaching. Since mechanical pulps are seldom well washed, most of the dissolved and colloidal substances are carried over to the wet end of the paper machine. Chemithermomechnical pulping (CTMP) results in the dissolution in process waters of up to 10% of the wood material, and sometimes even more. CTMP process waters contain essentially the same components as pulping and bleaching waters from TMP and other mechanical pulps, although in larger amounts. However, CTMP mills are mostly nonintegrated, and the pulp is extensively washed before delivery. Therefore, CTMP process waters do not occur in the further processing stages, namely production of fluff and tissue products, and paperboard. Also, chemical pulps and recycled pulps are usually thoroughly washed in the pulp mill and deinked, respectively, and do not carry over as much dissolved and colloidal material as mechanical pulps. However, beating of the pulps in the paper mill results in additional dissolution of fiber material.

Standard methods for measuring summative parameters have traditionally been predominant in analysis of papermaking process waters and effluents. However, such parameters provide little information on the true chemical character of the components. For a deeper understanding of the complex phenomena and mechanisms in papermaking chemistry, information of individual dissolved and colloidal components at the molecular level is necessary,

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demanding more sophisticated chromatographic and spectrometric techniques. Effluents discharged from paper mills should also be analyzed at the molecular level in order to achieve a thorough understanding of possible environmental effects. The traditional methods used in wood chemistry research are often too laborious and tedious for paper mill process analyses, where a large number of samples need to be analyzed in a reasonable time, and at a reasonable cost.

The emphasis of this chapter is on modern methods for the analysis of dissolved and colloidal substances in paper mill process waters and effluents. Methods are presented that provide information on individual components and component groups, but are still practical enough to be applied in industrial laboratories, and for paper mill process analyses. A recently developed practical integrated scheme for workup and analysis of paper mill pulp streams and process waters is described. Standard methods for process water and effluent analysis, and techniques for on-line process analysis are also briefly discussed.

Research methods needed for isolation and analysis of substances in paper mill waters are only briefly discussed, since these methods are treated in more detail in other chapters of this book.

9.2 Dissolved and Colloidal Substances

9.2.1 Material from Mechanical and Chemimechanical Pulps

Mechanical Pulps. In mechanical pulping and bleaching, 30–60 kg of wood material is dissolved or colloidally dispersed into the process waters for each ton of pulp produced. The main components of this material are hemicelluloses, pectic substances, lipophilic extractives (wood resin), lignans, and lignin-related substances. Alkaline peroxide bleaching causes degradation and additional dissolution of wood material, but some components dissolved in pulping are reprecipitated. Typical amounts of dissolved and colloidal substances released into waters from thermomechanical pulps (TMP) of spruce wood and in consecutive peroxide bleaching of the pulps are given in Table 9.1.

In mill practice, the amount and composition of dissolved and colloidal substances carried over to the paper machine is influenced by many process factors, such as quality of wood furnish, pulping process conditions, possible washing of the pulp, and the water system configuration, including the degree of closure of the mill. Coagulants such as alum or organic cationic polymers, so-called fixing agents, are commonly used in paper mills, especially to deposit colloidal material onto pulp fibers, resulting in cleaner process waters.

Table 9.1. Typical amounts of dissolved and colloidal substances in 1% suspensions of commercial TMP produced from Norway spruce (*Picea abies*). The pulp was bleached in the laboratory with alkaline hydrogen peroxide (3% on a dry pulp basis). Compiled from data of Holmbom et al. (1991), Thornton (1993), and Sundberg et al. (1994)

Constituents, kg/ton pulp	Unbleached TMP	Bleached TMP
Hemicelluloses	18	8
Galactoglucomannans	16	4
Xylan	2	5
Pectic substances	2	4
Lignin and related substances	7	11
Lignans	2	1
Lipophilic extractives (resin)	5	4
Acetic acid	1	20
Formic acid	0.1	4
Inorganic constituents	<1	5

Acetylated galactoglucomannan with a low galactose content is the dominating hemicellulose dissolved in mechanical pulping of softwoods. During the alkaline conditions of peroxide bleaching, an extensive deacetylation takes place and the acetic acid formed is the most abundant organic component in the waters from mechanical pulping (Samuelson and Sjöberg 1974; Holmbom et al. 1991; Thornton et al. 1994). Much of this deacetylated galactoglucomannan is readsorbed onto the fibers. Only small amounts of pectic substances are dissolved during mechanical pulping, but their amount is increased on peroxide bleaching and the methyl esters of galacturonic acid groups are hydrolyzed. The resulting ionized pectic acids account for a major part of the cationic polymer demand in the process waters from peroxide bleaching.

The lignin dissolved in mechanical pulping is structurally very similar to native wood lignin but has a weight-average molecular weight of only about 1500 Da (Pranovich et al. 1994). More lignin is dissolved in alkaline peroxide bleaching. This lignin contains carboxyl groups and its molecular weight is slightly higher than that of the lignin dissolved after mechanical pulping. Lignin-related aromatic oligomers are also dissolved in mechanical pulping waters.

Lignans comprise a complex group of phenolic extractives. Hydroxymatairesinol, existing in two stereoisomeric forms, is the main lignan type in the heartwood of Norway spruce. Lignans are largely oxidized by alkaline peroxide (Ekman and Holmbom 1989b). They are fairly water-soluble and can accumulate to high concentrations in highly closed water systems.

Wood resin (lipophilic wood extractives) forms the major part of the dispersed colloidal phase in mechanical pulp suspensions (Allen 1979). In softwood pulp suspensions, the colloidal resin droplets are composed mainly of

triglycerides, steryl esters, and resin acids. Smaller amounts of free fatty acids and sterols are also present. At pH above 6–7, resin and fatty acids are leached out from the colloidal resin droplets and the fibers into the water phase in the form of soaps. Alkaline peroxide bleaching leads to oxidation of dissolved resin acids of the abietadiene type, but the dispersed resin droplets are only slightly affected (Ekman and Holmbom 1989b).

Centrifuged process water samples also contain fiber fragments of colloidal dimensions, so-called micro-fines, which are debris of the fiber walls and the middle lamella (Thornton 1993).

Chemimechanical Pulps. Chemithermomechanical pulp (CTMP) is produced mostly in nonintegrated pulp mills. Although CTMP liquors do not normally become a part of papermaking operations, CTMP liquors are included in this chapter, and are not treated together with sulfite spent liquors in Chapter 7, because of their chemical similarity to TMP and other mechanical pulping waters.

In the production of peroxide-bleached CTMP from spruce, 5–15% of the wood material is dissolved or dispersed in the process waters. CTMP pulp is usually washed, and most of the washing water is discharged through an effluent treatment plant. As peroxide-bleached TMP waters, CTMP process waters mainly contain dissolved hemicelluloses, pectic acids, lignin (which is partially sulfonated), lignans, and low-molecular weight carboxylic acids, mainly acetic acid. Most of the resin is released from the wood into the process waters in CTMP mills (Karlsson et al. 1997) as dispersed colloidal droplets or as dissolved fatty and resin acid soaps. A low resin content is an important quality parameter of CTMP when used in fluff, tissue, and paperboard products and efficient resin removal is thus important.

9.2.2 Material from Chemical and Recycled Pulps

Chemical Pulps. Chemical pulps are extensively washed in pulp mills and relatively little dissolved and colloidal material is carried over to paper mills with chemical pulps. This is also the case when dry pulp in bales is used as raw material in nonintegrated paper mills.

However, some additional material is dissolved in the paper mill stock preparation, especially during beating of the pulp. It has been found that beating of bleached sulfite and sulfate pulps results in the dissolution of carbohydrates (0.3–0.6% of the dry pulp; Sjöström and Haglund 1963; Sjöström and Enström 1967). The dissolved carbohydrates were shown to consist almost completely of xylans, even in the case of softwood pulps where glucomannan is the dominant hemicellulose in the pulp. In fact, of the xylan present in the unbeaten pulp, a maximum of 20% was dissolved during beating. Similar observations were made later, which showed that suspensions of typical

Finnish ECF- and TCF-bleached softwood kraft pulps (mainly pine) contained 0.14–0.21% dissolved hemicelluloses on pulp basis after laboratory beating, 70–80% of which was xylans (Holmbom 1996).

Of course, practically no lignin, or much less than 0.1%, is present in fully bleached chemical pulps. The wood resin content is also very low, in bleached kraft pulps it is usually less than 0.1%. However, in the case of unbleached chemical pulps, some residual lignin and extractives are dissolved from the pulp into the wash solution (Wilcox and Goring 1990).

Recycled pulps. Modern deinking processes, which include efficient washing stages, will remove most of the material released into process waters from the recycled paper. Thus, recycled deinked pulps do not carry over much dissolved and colloidal substances to papermaking operations. Deinked pulp suspensions contain mainly dissolved carbohydrates, especially xylans, and dispersed resin components (Holmbom 1997). Furthermore, recycled and deinked pulps are usually contaminated with residuals of adhesives, coating binders, starch derivatives, and deinking chemicals, which can give detrimental deposits of "stickies" in papermaking.

9.2.3 Non-Wood Components

A wide variety of process chemicals are used in papermaking. Chemicals added at the wet end will remain to some extent in process waters, depending on their retention in the paper web. These wet-end chemicals include retention and fixing aids, wet-strength and hydrophobation agents, bactericides, etc. They represent compounds of varying chemical compositions, such as modified starches, synthetic polymers, and alkyl ketene dimers (AKD). Chemicals and coating agents added at the dry end of the paper machine for hydrophobation or for other surface-improving purposes, will partly circulate back to the wet end in connection with a paper broke. The broke plays a significant role at the wet end, especially in the production of coated paper. Coated broke brings latex polymers into the wet end. The polymers are known to interact with wood resin to form troublesome deposits, so-called white pitch. Coated broke also often carries much soluble starch, which can significantly increase the total amount of dissolved material at the wet end. In the case of recycled paper, additional chemicals used in converting, printing, and deinking, are introduced to the system. Analytical techniques for all these paper chemicals, although of great interest and significance, are outside the scope of this book.

Finally, it should be mentioned that raw waters can contain considerable amounts of impurities, including humic substances and inorganic salts, but usually only purified water is used in paper mills.

9.3 Separation, Fractionation, and Analysis

9.3.1 Standard Determinations of Summative Parameters

Analyses of process waters and effluents in paper mills are still today predominantly based on methods for summative parameters. Of the wide variety of determinations available, the most common are listed in Table 9.2. Standard methods have been developed by ISO, SCAN, TAPPI, CPPA, and APPITA. Although these parameters are useful for process control, they lack compound specificity and do not provide information about individual components.

9.3.2 Preparative Separation of Dissolved and Colloidal Substances

Besides dissolved and colloidal substances, paper mill process waters and effluents contain varying amounts of fibers, fines, fillers, and various aggregates. There are no sharp boundaries, either between fines, filler particles, and colloidal particles, or between colloidal particles and dissolved macromolecules. However, fractionation, as shown in Fig. 9.1, can provide well-defined

Table 9.2. Common determinations for paper mill waters

Parameter	Main components	Measurement principle
Total dissolved solids	Organic and inorganic compounds	Evaporation, gravimetry
Chemical oxygen demand (COD)	Organic compounds	Dichromate oxidation
Biochemical oxygen demand (BOD)	Easily degradable organic compounds	Biochemical oxidation by bacteria in sludge
Total organic carbon	Organic compounds	Catalytic oxidation, carbon dioxide determination
Ash	Inorganic compounds	Combustion, gravimetry
Conductivity	Electrolytes (salts)	Electric conductivity
Cationic demand	Anionic polymers	Titration with cationic polyelectrolyte
Turbidity	Dispersed particles	Light scattering
Total extractives	Lipophilic compounds	Extraction, gravimetry
Total carbohydrates	Mono-, oligo-, and polysaccharides	Colorimetry with orcinol

fractions of dissolved and dispersed components in paper mill waters for further chemical analysis.

Water samples, containing mainly the dissolved and colloidal substances, can be prepared from fiber suspensions and process waters in a reproducible manner by sedimentation, or preferably by centrifugation (Thornton 1993). In contrast, ordinary filtration gives variable losses of colloidal components because of trapping in the fiber mat formed. Samples of paper mill process waters with pulp consistencies up to about 4% are immediately, while still warm, centrifuged at 1500 rpm (500 g) for 30 min. The supernatant, containing the dissolved and colloidal substances, should be pipetted off with care, avoiding redispersion of sedimented fiber and filler material. Samples containing only dissolved substances can be prepared from those containing dissolved and colloidal substances by filtration through membranes with 0.1–0.2 μm pore diameters. If larger particles than colloidal ones are of interest, filtration in a

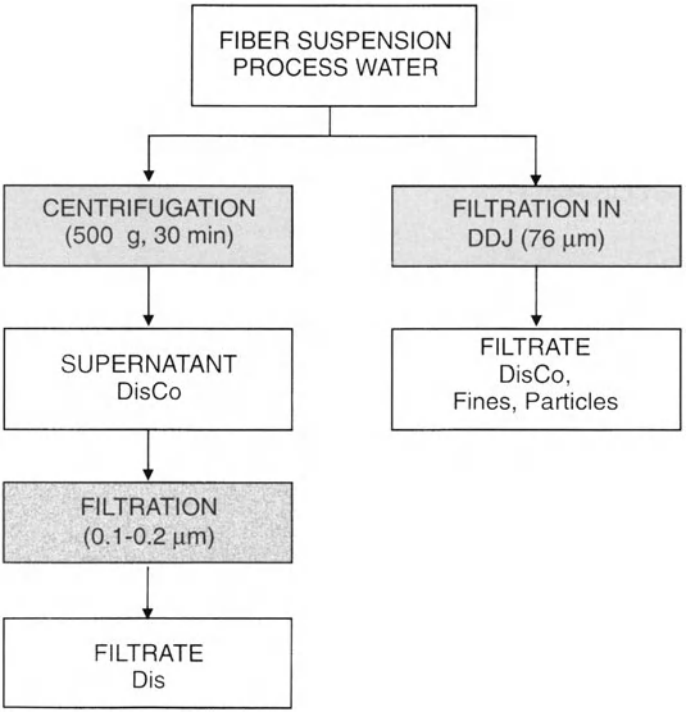

Fig. 9.1. Scheme for isolation of samples containing dissolved and colloidal substances and larger dispersed particles from fiber suspensions and process waters. *DDJ* dynamic drainage jar (also called Britt jar). *DisCo* Dissolved and colloidal substances. *Dis* Dissolved substances. *Fines* Fibers passing the 200 mesh wire (74 μm) *Particles* Non-fiber particles, such as fillers and aggregated resin or latex particles

dynamic drainage jar (DDJ), equipped with, e.g., a 200 mesh wire (holes ≈74 μm) can be performed, giving a suspension containing dissolved and colloidal substances as well as larger dispersed particles, such as fines, fillers, and aggregates of resin and latex. Fractionation into high- and low-molecular weight fractions can be performed by ultrafiltration with appropriate membrane filters (Sjöström and Ödberg 1994).

9.3.3 A Practical Integrated Analytical Scheme

An integrated analytical scheme has recently been developed, according to which dissolved and colloidal wood-derived substances present in paper mill process waters and effluents can be separated and analyzed in a practical manner (Fig. 9.2). The scheme includes removal of fibers and fillers by centrifugation, followed by extraction and GC analysis of both lipophilic and hydrophilic extractives, as well as analysis of hemicelluloses and pectins via acid methanolysis and GC. The total amount of dissolved lignin-like substances is determined by UV spectrophotometry at 280 nm. Standard measurements, such as pH, TOC, conductivity, turbidity, and cationic demand can be a valuable supplement to the component analyses.

9.3.4 Analysis of Carbohydrates

Carbohydrates, composed primarily of various hemicelluloses, pectins, and starch, form the major part of the dissolved substances in paper mill waters. Mono- and disaccharides are also present, but in very low concentrations.

Methanolysis – GC. Acid methanolysis followed by GC analysis (Chap. 3) has been found to be very useful for determination of dissolved carbohydrates in paper mill waters (Holmbom and Örså 1993). Small samples of water, normally 2 ml, are first freeze-dried. Effective cleavage of hemicelluloses into their monomeric units, in the form of methyl glycosides, is achieved by acid methanolysis with a solution of 2 M HCl in anhydrous methanol. Samples are kept for 3 h in an oven at 100 °C. Uronic acid units, which are extensively degraded on acid hydrolysis, are much better preserved in methanolysis because the carboxyl groups are methylated, making them less susceptible to degradation. The hydrolysis-resistant bond between xylose and 4-*O*-methylglucuronic acid in xylans is also cleaved to a high extent, and the methyl glycosides of both xylose and 4-*O*-methylglucuronic acid methyl ester are obtained in fairly good yield.

The monosaccharides (as methyl glycosides) obtained by methanolysis are converted to trimethylsilyl ethers, using the reagents hexamethyldisilazane

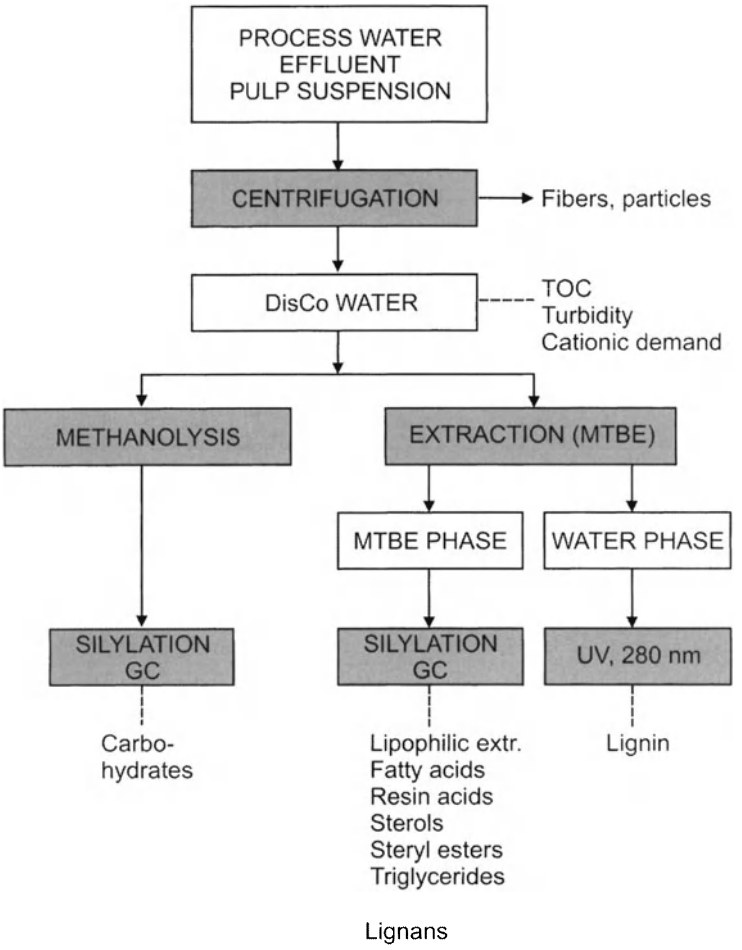

Fig. 9.2. An integrated scheme for analysis of the main components in paper mill process waters and effluents. (Holmbom and Örså 1993)

(HMDS) and trichloromethylsilane (TMCS), and are analyzed by GC on a non-polar capillary column. All monosaccharide peaks of interest are separated by GC analysis on a 25–30 m standard dimethylpolysiloxane capillary column, although direct silylation leads to 2–5 peaks for each monosaccharide (Fig. 9.3). Multiple peaks can be easily handled by modern computer-based integrators. The pattern of multiple peaks is specific for each sugar and actually facilitates peak identification.

Sorbitol is a suitable internal standard for quantification, and is preferably added after the methanolysis step. For quantitative calibration purposes, parallel analyses should be made with reference samples containing known amounts of pure monosaccharides or polysaccharides.

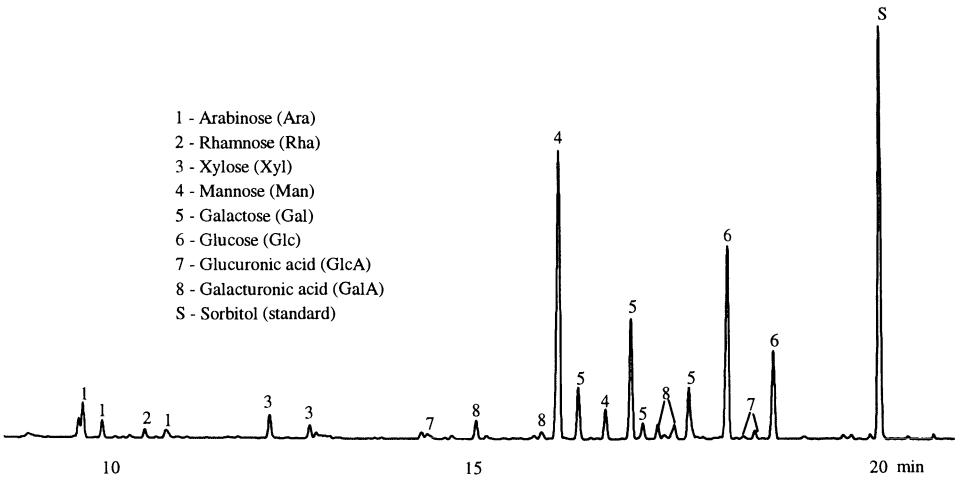

Fig. 9.3. A typical gas chromatogram of carbohydrates after methanolysis and silylation. Sample: wire water from a paper machine producing SC magazine paper from dithionite-bleached spruce TMP and bleached softwood kraft pulp. Column: dimethyl polysiloxane, 25 m/0.20 mm/0.11 μm, programmed 100–240 °C at 4 °C/min

The method provides simultaneous determination of both neutral and acidic sugar units. Several samples can be analyzed in parallel, making the method quite convenient, especially when the GC analysis is automated. Recalculation of the values obtained to polysaccharides (hemicelluloses) is based on the knowledge (or assumption) of the proportions of the respective monomeric units (Chap. 3).

Mono- and disaccharides can be determined separately by GC analysis directly of freeze-dried samples without acid methanolysis, after derivatization to trimethylsilyl ethers or as alditol acetates (Chap. 3).

Other Methods. Hydrolysis with strong acids (usually H_2SO_4) is traditionally used in the analysis of carbohydrates in wood and pulps and it results in a complete (or nearly complete) cleavage of the glycosidic bonds between the monosaccharide units (Chaps. 1 and 3). However, as already mentioned, acid hydrolysis cannot be used to liberate the uronic acid groups from the xylan backbone.

Mixtures containing various cellulolytic and hemicellulolytic enzymes have been used in the analysis of kraft pulps (Tenkanen et al. 1995). An important advantage in the case of kraft pulps is the preservation of the hexenuronic acid units that are destroyed on acid hydrolysis or methanolysis. Enzymatic cleavage may also be useful for analysis of polysaccharides in process waters and effluents.

Other chromatographic and spectroscopic techniques than GC have been used for analysis of carbohydrates in process waters. Sugar monomers

obtained by hydrolysis of polysaccharides with acids or enzymes have successfully been separated by HPLC using anion exchange resins and pulsed amperometric detection (Sullivan and Douek 1994; Tenkanen et al. 1995). Pyrolysis-GC/MS has been used to characterize and estimate the relative amounts of polysaccharides and aromatic lignin-related components in paper mill process waters (Sjöström and Reunanen 1990; Kleen and Lindström 1994).

For detailed structural characterizations, different types of hemicelluloses need to be isolated by appropriate preparative procedures. These methods are usually tedious, and are not very practical for mill process analyses involving a large number of samples. Anionic hemicelluloses and proteins can be isolated by use of ion exchange resins (Sjöström 1990). Size-exclusion chromatography (SEC) in buffered water systems can provide information about the molecular weights of polysaccharides.

9.3.5 Analysis of Extractives

Both lipophilic and moderately hydrophilic extractives in paper mill waters can be determined by appropriate solvent extraction combined with analysis by GC. Methyl *tert*-butyl ether (MTBE) is an effective solvent for extraction of both lipophilic extractive groups and the more hydrophilic lignans, which are abundant in mechanical pulping waters (Voss and Rapsomatiotis 1985; Örså and Holmbom 1994). A mixture of hexane and acetone or ethanol (2:1 v/v) is selective for lipophilic extractives, giving practically no extraction of lignans. If only lipophilic extractives are of interest, hexane-acetone/ethanol can be recommended as extraction solvent. For convenience, extractions can be performed with small water samples (normally 4 ml) in test tubes with several samples in parallel. Adjustment of the acidity to pH $\approx$3 prior to extraction ensures good extraction of lignans. Two extractions are sufficient to achieve high recovery of lipophilic extractives, but three extractions are needed to obtain satisfactory yields of lignans. Vigorous shaking is essential for high extraction yields of lipophilic extractives, because the extractives occur mainly in the form of colloidal droplets.

Solid-phase extraction using either reversed-phase or cross-linked polystyrene adsorbents is an alternative to solvent extraction for preparative isolation of lipophilic components from waters. Solid-phase microextraction (SPME) is a convenient extraction technique utilizing a small polymer-coated fused-silica fiber. SPME also has a potential for extraction of paper mill waters, although it has not yet been evaluated for this application. Successful analyses of both short-chain and long-chain fatty acids by SPME has recently been reported (Pan and Pawliszyn 1997).

Lipophilic extracts can be analyzed for their component groups by GC on short (5–7 m) columns with a thin film of nonpolar stationary phase (Sitholé

et al. 1992; Örså and Holmbom 1994). This technique enables convenient determination of the main extractive groups, including fatty acids, resin acids, lignans, sterols, steryl esters, and triglycerides (Fig. 9.4). Special features of this method are: the use of several relevant internal standard compounds, automated injection, on-column injection instead of split injection, and use of advanced systems for integration of chromatograms. Silylation enables simultaneous derivatization of fatty and resin acids, lignans, and sterols. The GC analysis is described in more detail in Chapter 5 and in the paper by Örså and Holmbom (1994). If detailed information on the individual fatty and resin acids, lignans, and sterols is needed, the same extract can be analyzed on a standard 15–25 m long GC column (Ekman and Holmbom 1989a).

SEC in tetrahydrofuran can also be used for separation of different lipophilic component groups (Chap. 5).

9.3.6 Analysis of Lignin and Other Aromatic Components

An estimate of the total amount of aromatic lignin-related components in paper mill waters can be obtained by UV spectrophotometry at 280 nm. However, water samples should first be extracted to remove the strongly UV-absorbing lignans and the lipophilic extractives, using multiple extraction at a

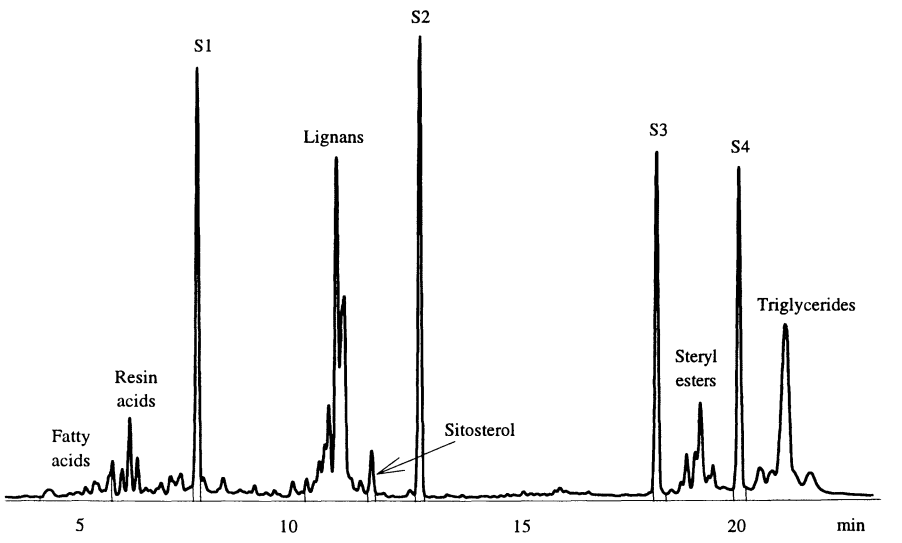

Fig. 9.4. A gas chromatogram of a silylated MTBE extract. Sample: press section water from the same SC paper machine as in Fig. 9.3. Column: dimethyl polysiloxane, 5 m/0.53 mm/0.15 µm, programmed 100–340 °C/min at 12 °C/min. Peaks *S1–S4* are from internal standards

low pH with MTBE as discussed above. Only small amounts of other aromatic components of higher-molecular weight (lignin and lignin-like oligomers) are removed by this technique (Örså and Holmbom 1994) Removal of lipophilic extractives also eliminates disturbance due to colloidal resin droplets. A residue of MTBE does not interfere, since it does not absorb at 280 nm. Quantitative calibration can be made by using a pure sample of spruce milled-wood lignin (MWL) dissolved in methanol-water (80:20 v/v). The absorptivity of lignin isolated from mechanical pulping liquors does not significantly deviate from the absorptivity of MWL.

Soluble lignins can be isolated using crosslinked polyvinylbenzene or polyacrylate resins (XAD-4 or XAD-8; Schmitt et al. 1990; Sjöström 1990). SEC in tetrahydrofuran can be used for determination of the molecular-weight distribution of lignins.

9.3.7 Analysis of Miscellaneous Pulp Components, Including Inorganics

Besides carbohydrates, extractives, lignins, and lignin-related compounds, low-molecular weight organic acids, traces of proteins, and inorganic ions are released from pulps into paper mill waters. Acetic acid, formic acid, and other low-molecular weight carboxylic acids can be analyzed by GC after converting them to benzyl esters (Chap. 7). Inorganic components, such as calcium, aluminum, and iron ions can play an important role in paper mill systems, due to their coagulating effect on colloidal particles and their effect on paper brightness. Large amounts of silicates are introduced on peroxide bleaching of mechanical pulps. Polymerized silicates, but not soluble oligomeric forms, can give disturbing interactions with paper process chemicals, especially with cationic coagulants. The total amount of silicates can be determined by atomic absorption spectrophotometry (AAS), and the soluble silicates as molybdic complexes by UV spectrophotometry. The difference corresponds to the detrimental polymeric silicates (Saastamoinen et al. 1996). For the analysis of inorganics, see Chapter 10.

9.3.8 Analysis of Non-Wood Components

In practice, paper mill waters may also contain a whole array of paper chemicals, such as fixing and retention aids, defoamers, sizing agents, dry- and wet-strength aids, biocides, etc. Most of these occur at low concentrations compared to the soluble and colloidal substances released, especially from mechanical pulps.

Modified starches are largely used in papermaking as retention and fixing chemicals, dry strength agents, surface sizing agents, and as binders in pigment

coating. An estimate of the amount of starch can be made from carbohydrate analysis by methanolysis and GC, based on the amount of glucose, after subtracting hemicellulose-based glucose. Analytical procedures have been developed, e.g., for sizing agents such as AKD and ASA which can be determined by extraction and GC analysis. However, sensitive and selective methods are still lacking for many common paper chemicals.

Coated broke and recycled paper cause introduction of disturbing amounts of synthetic polymers. Much work has been done to develop analytical techniques for these polymers. An inherent problem is their limited solubility, particularly if the polymers are cross-linked. Soluble polymers can be analyzed by SEC in combination with IR spectroscopy or pyrolysis-GC (Sitholé and Allen 1994; Holmbom 1996).

9.4 On-Line Process Analysis

Continuous process control is the key to consistent runnability and paper quality in paper mills. Control of the wet-end chemistry is particularly important in this respect. On-line process measurements at the wet end are thus urgently needed.

Determination of concentrations and flows of fibers, fines, and fillers is of particular importance in papermaking wet end control. Systems for their determination have recently become commercially available (Guest 1996). However, along with the trend toward water system closure, analysis of dissolved and colloidal components in various paper mill process streams is becoming ever more important.

The first step for on-line measurement of dissolved and colloidal components is some kind of prefractionation to remove the main part of the fibers and fillers. Various filtration systems have been developed and some of them are also in continuous use in paper mills.

Measuring systems for on-line determination of certain summative parameters, such as dissolved organic carbon (DOC), turbidity, conductivity, and anionic charge (cationic demand), are already in use in some paper mills. However, except for some metal cations, on-line methods are still lacking for specific dissolved components or component groups. This is not possible with individual sensors but requires advanced process analytical systems, including several techniques. Especially, there is a need for on-line analysis of strongly interfering components, such as the highly anionic pectic acids, dispersed wood resin, and other stickies. Techniques based on sequential injection analysis (SIA), continuous-flow extraction and flow cytometry show promise in meeting these needs (Ivaska and Ruzicka 1993; Kröhl et al. 1994; Rice et al. 1997).

9.5 Summary

The chemical interactions at the wet end of papermaking, involving dissolved and colloidal substances, paper chemicals, various fibers, and fillers, are of key importance in papermaking, both with respect to paper machine runnability and paper quality. Only with the aid of specific analysis of the participating substances, will it be possible to fully understand the mechanisms of these complex interactions. Such molecular-level analyses are also important in order to fully understand the various environmental effects of effluent components.

A wide variety of analytical techniques can be used for analysis of components in paper mill waters; in principle most of the methods described in this book. However, only rapid and practical methods can be used for process analyses in paper mills, usually involving a large number of samples.

A practical integrated analytical system has been devised that enables convenient and reasonably detailed quantitative determination of various dissolved and colloidal wood components in paper mill process streams and effluents. Water containing the dissolved and colloidal substances are separated from fibers and other non-colloidal particles by centrifugation. The supernatant obtained is analyzed for carbohydrates, extractives, and lignin. The amount and composition of carbohydrates present, mainly as hemicelluloses and pectic substances, is determined by GC after methanolysis. Both lipophilic components and lignans can be extracted with methyl *tert*-butyl ether (MTBE). The extractives are subsequently analyzed by GC for determination of free fatty and resin acids, sterols, steryl esters, triglycerides, and lignans. Dissolved lignin and lignin-like substances are determined from the UV absorbance at 280 nm of the extracted water sample. The method enables convenient analysis of several water samples in parallel. A sample of less than 10 ml is required for component analyses.

Practical methods for on-line process analysis of specific components and component groups are urgently needed, both for process control of the wet end of papermaking and for continuous monitoring of effluents.

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10 Analysis of Inorganic Constituents

A. Ivaska and L. Harju

10.1 Introduction

The inorganic components in wood are various salts of carbonates, silicates, phosphates, and oxalates (predominantly in bark, Chap. 1). They are minor constituents of the wood material and their total concentrations (measured as ash) rarely exceed the 1% level. The inorganic ions are normally taken up by the living tree through the roots from the soil. The mineral elements play an important role in the growing process of the tree. The modes of uptake and distribution of the different elements in the living tree are largely unknown. The inorganic compounds in the wood exist as mineral salts and as cations bound to acidic groups. Information about concentrations of a number of elements in different wood species and their location in the tree have been reported in the literature (Young and Guinn 1966; Koch 1972, 1985, 1996; Wardell and Hart 1973). Table 10.1 shows the distribution of the elements in different concentration ranges in the trunk wood of a pine tree from southwestern Finland. The concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS).

Ion exchange is assumed to be the main mechanism for sorption of metal ions to wood and pulp (Sjöström et al. 1965; Bryant and Edwards 1996; Eriksson and Grén 1996; Abubakr et al. 1997). The binding sites are mainly carboxylic acid groups. Their number is normally rather low and varies in pulps depending on the pulping and bleaching methods used. Metal ions bound to functional groups in wood and pulp can usually be desorbed by washing with acid, or using chelating agents such as EDTA and DTPA.

The inorganic constituents found in the different process steps in a kraft pulp mill mainly originate from the raw material, make-up chemicals, process water, and corrosion from the process equipment. Many of the inorganic components can detrimentally affect both the pulping and the bleaching processes as well as the quality of the final pulp and its further processing. For example, Mn, Fe, and Cu are known to be harmful in totally chlorine-free (TCF) bleaching (Sjöström and Välttilä 1978; Heijnesson et al. 1995).

By combination of different analytical techniques, speciation studies can be made. Nyman and Ivaska (1993) have studied different species of calcium in the white water of a paper machine. The effect of a pulp and paper mill on metal concentrations in the receiving lake system has been investigated by Holmbom et al. (1994).

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Table 10.1. Elemental distribution in different concentration ranges for stem wood of a Scots pine (*Pinus sylvestris*) tree

Concentration range, ppm (mg/kg)	Elements
1000–100	Ca, K, Mg
100–10	F, Fe, Mn, Na, P, S
10–1	Al, B, Si, Sr, Zn, Ti
1–0.1	Ag, Ba, Cd, Cr, Cu, Ni, Rb, Sn
0.1–0.01	Bi, Br, Ce, Co, I, La, Li, Pb, Se, W
0.1–0.001	As, Eu, Gd, Hf, Hg, Mo, Nd, Pr, Sc, Sb

The total inorganic components in wood, pulp, and paper are often collectively determined as the ash content. The ash can further be subdivided into acid-soluble and acid-insoluble ashes, the latter being mainly composed of silicates. Standard methods for determination of inorganic constituents in wood, pulp, and paper, as well as in pulping liquors have been devised (TAPPI Test Methods 1996–1997). The recommended standard methods describe in detail the sample preparation and the analytical procedures. The most common standard analytical methods include potentiometric, complexometric, and redox titrimetry, as well as spectrophotometry and atomic absorption spectroscopy. The development of analytical methods based on instrumentation has been very rapid (Willard et al. 1988; Skoog and Leary 1992), and many modern instrumental techniques are rather sophisticated and are not yet used as standard methods.

Generally, atomic absorption spectroscopy (AAS), plasma procedures, various X-ray techniques, and ion chromatography (IC) constitute the key methods for determination of inorganic components in wood-related materials. Electron spectroscopic methods such as electron spectroscopy for chemical analysis (ESCA) are powerful tools for studying surfaces. Combinations of different analytical techniques are needed to increase selectivity and sensitivity. An example of such a combined technique is inductively coupled plasma-mass spectrometry (ICP-MS).

Introduction of computers to analytical instrumentation has increased the degree of automation and control of the different functions of the instruments. Computers also allow the use of advanced mathematical methods both in formation of the excitation signal and in the final analysis of the experimental data.

The importance of the determination of the inorganic compounds in the pulp and paper industry has increased, and adequate analytical methods are needed. The purpose of this chapter is to describe the most common instrumental analytical methods used today for determination of inorganic constituents in wood, pulp, and paper, and also to describe the possibilities that some other, more advanced analytical techniques can offer.

10.2 Sampling and Sample Preparation

Wood and pulps are heterogeneous materials and special attention should be paid to sampling in order to get a representative sample. Koch (1972, 1985, 1996) and Hakkila (1989) describe variations in concentrations of different elements among, and within, various North American tree species. Different parts of a tree have been demonstrated to contain widely different concentrations of elements. The following order of decreasing content of inorganic constituents has been reported within a tree (Young and Guinn 1966): bark, tiny roots, twigs, roots, branches, and stem. An increase in the mineral element content from the outer edge of the sapwood to the heartwood and to the pith has also been found (Fossum et al. 1972). A strong seasonal variation of element content within annual growth rings of trees has also been reported (Harju et al. 1996).

After sampling, the material has to be ground to a suitable small particle size (<0.4 mm) using a Wiley mill, a hammer-type mill or a rotory-knife cutter. After pulverization, the material is homogenized. In the laboratory the samples are oven-dried at 105 °C or freeze-dried to determine the moisture content of the sample in order to express final concentrations on a dry matter basis.

For the wet-chemical methods, the sample has first to be dissolved. This is the most time-consuming step in the whole analytical procedure. Complete dissolution of a sample is often rather difficult to achieve. The dissolution process to be used in a particular assay is determined by the type of sample to be analyzed and the inorganic constituents to be determined. Wet digestion of samples usually involves the use of strong acids like HNO_3, HCl, H_2SO_4, $HClO_4$, or HF, or various mixtures of them (Bock 1979). A simple and effective technique is to digest the sample with acid in a volumetric flask at elevated temperatures with a reflux condenser. Nitric acid is quite effective in digestion of biological materials, and hydrogen peroxide can additionally be used to complete the oxidation process. However, open vessels can involve risks for contamination and loss of volatile elements. To avoid these problems, the dissolution may be performed in closed bombs of polytetrafluoroethylene (PTFE) (Uhrberg 1982).

An effective and rapid dissolution of biological samples can be obtained by the microwave digestion technique (Nöttner et al. 1990; Anonymous 1995). For digestion of wood and cellulose samples, a 6:1 mixture of nitric acid (65%) and hydrogen peroxide (30%) has been recommended (Anonymous 1995). The method requires approximately 500 mg of sample and the whole digestion procedure takes about 30 min. Due to the efficiency of the microwave technique, only small amounts of acids are used for dissolution and the risk of contamination from reagents is minimized. This results in low background values which are necessary for reliable determination of elements at trace and ultra-trace concentration levels, i.e., down to ppt (ng/l) level. Safety prescriptions

must be carefully followed when microwave acid digestion is used. This is especially important in digestion of organic materials at high temperatures and pressures.

For determination of halogens, sulfur, phosphorus, and boron, it is recommended to dissolve the sample in a Schöniger flask in the presence of oxygen (Bock 1979). The Schöniger technique is especially useful prior to IC determination. The solid sample is combusted in a conical flask filled with oxygen and the combustion products formed are absorbed in a proper solution. The choice of the absorption solution depends on the elements to be determined. Deionized water or dilute KOH or NaOH solutions are often recommended for determination of halogens and a 3–6% H_2O_2 solution for determination of sulfur.

The sensitivity of the assay can further be increased by dry ashing of the original sample at 550 °C. TAPPI (TAPPI Test Methods 1996–1997) recommends 575 ± 25 °C for ashing of wood specimens. With wood samples having a typical ash content of 0.2–0.5%, a preconcentration factor as high as 200–500 may be obtained when dry ashing is employed (Harju et al. 1997). Dry ashing also allows the use of large quantities of sample, up to 100 g, and is therefore an advantageous approach in analyzing such a heterogeneous material as wood. Large sample size reduces the errors that might arise from the inhomogeneity of the sample. Pellets of the ash can also be pressed if analytical methods for solid samples are used. A drawback with dry ashing is that some volatile elements such as halogens, mercury, and lead might be lost.

10.3 Spectroscopy

Spectroscopic methods are based on the interaction of electromagnetic radiation with the material studied. Depending on the technique used, either absorption or emission of radiation is measured. Absorption and emission are associated with the energy states of the material and can be used both for qualitative and quantitative determinations.

10.3.1 Atomic Absorption Spectroscopy

In AAS, a liquid sample is aspirated into a nebulizer where it is transformed into droplets, forming an aerosol. The fine mist is then brought together with a mixture of fuel and oxidizer. This gaseous mixture is further transferred into a burner. The temperature of the flame is between 2000 and 3000 °C depending on the composition of the fuel/oxidant mixture. Due to the elevated temperature, the solvent is evaporated and the sample is atomized. The excitation source in AAS is a hollow-cathode lamp. The lamp is filled with an inert gas,

usually argon or neon, and contains two electrodes, an anode and a cathode. The cathode is made of the element to be determined. By applying a high voltage between the electrodes, an electrical discharge is created, resulting in emission of an electromagnetic radiation characteristic of the cathode material. The radiation is directed through the flame and then via a monochromator to a detector. If the sample, introduced into the flame, contains the same element as the cathode, the atoms in the flame will absorb that radiation and a decrease in its intensity will be observed. Absorption of radiation follows the Lambert-Beer's law and the concentration of the element of interest in the sample can therefore be determined. In AAS, each element needs a lamp of its own, which has to be changed when a new element is to be determined. There are also combination lamps that can be used for determination of a number of different elements. In these lamps the cathode material consists of the elements for which the lamp has been designed.

Atomization of the sample can also be done electrothermally in a graphite furnace (GFAAS), where the flame is replaced by a horizontally placed graphite tube. The sample is pipetted into the tube through a small hole in the wall. When an electrical current is passed through the graphite tube, the temperature in the tube will increase. First the solvent is evaporated, and upon further increase in temperature, the sample will be ashed and atomized. Radiation from the hollow-cathode lamp is directed through the tube and then via the monochromator to the detector. Because atomization of the sample takes place in a closed tube, the concentration of the sample atoms will be higher in the tube than in the flame. This results in a higher sensitivity of the GFAAS method than that of the AAS method with a flame. A schematic drawing of a GFAAS instrument is shown in Fig. 10.1. The light sources are the different hollow cathode lamps, one for each element. The sample is introduced into the graphite tube through a hole in the side of the tube. The wavelength separation is done with an Echelle grating monochromator.

Mercury is difficult to determine by the regular AAS technique, due to the high volatility of this metal. Determination of mercury is most commonly done by a "cold vapor" method. The aqueous sample containing mercury is placed in a closed container. Solution of a strong reducing agent, such as tin(ll)chloride, is then added to the solution. Mercury ions in the solution are reduced to metallic mercury, which is purged from the solution with argon or nitrogen. The purged gas is directed into a flow-through quartz cuvette. Radiation from a mercury lamp is directed through the same cuvette and absorption of the radiation will take place if the sample contains mercury.

10.3.2 Plasma Methods with Atomic Emission Spectroscopy

At high temperatures (>6000 °C), an inert gas such as argon will be ionized and forms a plasma containing ions and electrons. Plasma is sometimes called

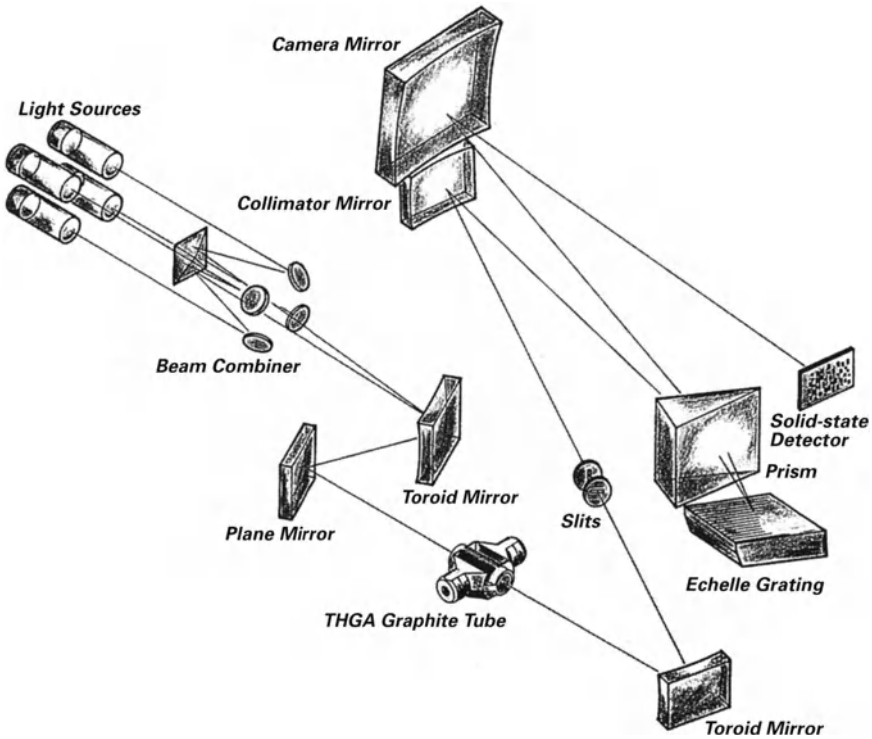

Fig. 10.1. Schematic drawing of a GFAAS instrument. (Anonymous 1994)

the fourth state of material. When an external electromagnetic field is applied to the electrons generated in the plasma, the plasma torch can be kept glowing after the initial firing and formation of the plasma. By collisions between electrons and the gas atoms, the energy is evenly distributed in the plasma. The temperature in the plasma may be as high as 8000–10000°C. The sample is introduced into the plasma through a nebulizer where it is transformed into an aerosol, which is then transported into the plasma with an argon stream. Due to the high temperature of the plasma, practically all the molecules of the sample will be atomized and transferred to the excited state. When the energy of excited atoms are returned to the ground state, radiation is emitted. The emission signal from the plasma is measured with an atomic emission spectrometer (AES). All elements have their own characteristic wavelengths of emission radiation. The sensitivity of the different wavelengths of a certain element varies, and the measurement is normally done at the most sensitive wavelength. The intensity of the emission signal depends on the concentration of the element in the plasma. There are two commercial types of plasma sources available: direct current plasma (DCP) and inductively coupled plasma (ICP).

In the case of DCP, the high temperature necessary for formation of the plasma, and for sustaining it, is produced by a DC-arc. The electrical discharge takes place between one tungsten cathode and two graphite anodes. Both anodes are cooled with extra argon flows. The electrodes are placed so that the arc formed has a inverted Y-shape. Plasma is formed in the spot where the two branches of the arc intersect. This experimental arrangement results in an increased temperature in the plasma. The sample aerosol is introduced directly into this high temperature zone.

The heart of an ICP instrument is the part where the plasma torch is formed. It consists of three concentric quartz tubes. An induction coil is placed around the top of the outer tube, and an argon flow is introduced radially between the middle and the outer tube. The plasma is ignited by a high voltage discharge and is sustained by the high power and high frequency magnetic field from the induction coil. The sample is introduced through the inner tube. Another argon flow is also introduced into the outer tube. The purpose of this flow is to cool the quartz tubes and to give the right shape to the plasma. The layout of a typical ICP-AES instrument is shown in Fig. 10.2.

The ICP-MS instrument contains a plasma part where the sample is ionized. The ions from the plasma are introduced into a mass spectrometer, where they are separated from each other based on their different charge/mass ratio. Under normal experimental conditions, the atoms have one positive charge

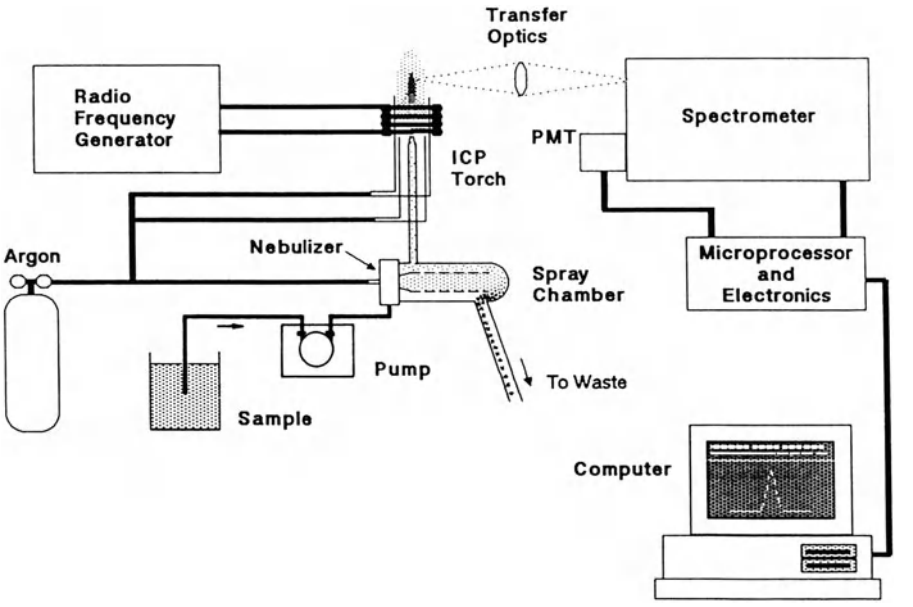

Fig. 10.2. Major components and layout of a typical ICP-AES instrument. (Boss and Fredeen 1989)

and therefore the separation will be based on the different masses of the species. The result is a mass spectrum with peaks at different mass numbers. The intensity of a peak is a measure of the concentration of that element in the sample. Most of the elements have several natural isotopes, and it is thus possible to select a peak where interferences from other elements are minimal. A schematic drawing of an ICP-MS instrument is shown in Fig. 10.3 and a mass spectrum in Fig. 10.4.

Table 10.2 shows some analytical data determined by ICP-MS for a sample of pine kraft pulp taken after the first washer after the digester. The sample was dried and digested in HNO_3 and H_2O_2 by the microwave digestion technique before the analytical determination. Results of an ICP-MS analysis of a process liquor sampled after the digester are given in Table 10.3.

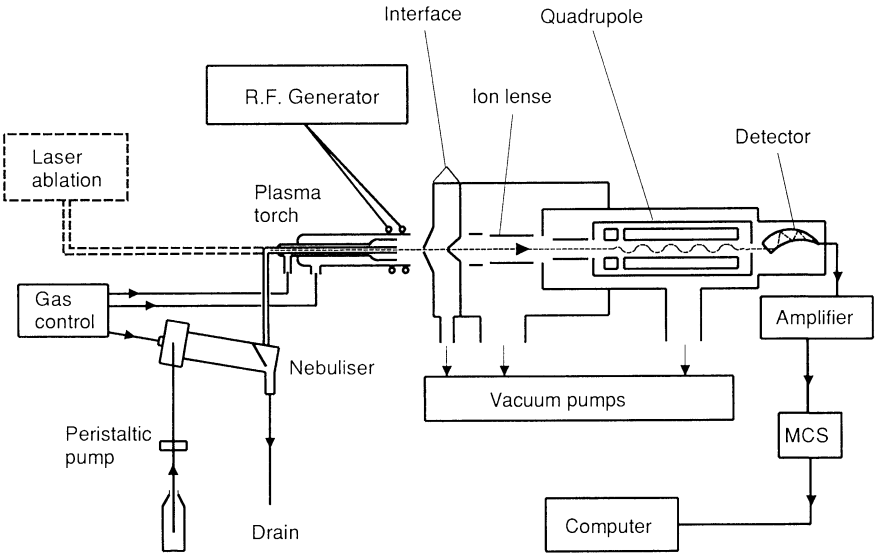

Fig. 10.3. Schematic presentation of a LA-ICP-MS instrument

Table 10.2. Concentration (ppm or mg/kg) of different elements in a pine kraft pulp sample after the digester, determined by ICP-MS

Li	Mg	Al	P	Ca	Ti	V	Cr	Mn	Fe
0.015	332	31.2	41.7	1600	1.09	0.08	1.01	31.3	76.7
Co	Cu	Zn	Ga	As	Rb	Sr	Zr	Mo	Ag
0.013	13.5	24.3	0.73	0.40	0.035	5.33	0.83	0.075	1.66
Cd	Sn	Ba	La	Ce	Hg	Pb	Bi		
0.082	0.23	15.0	0.023	0.057	0.39	0.63	1.19		

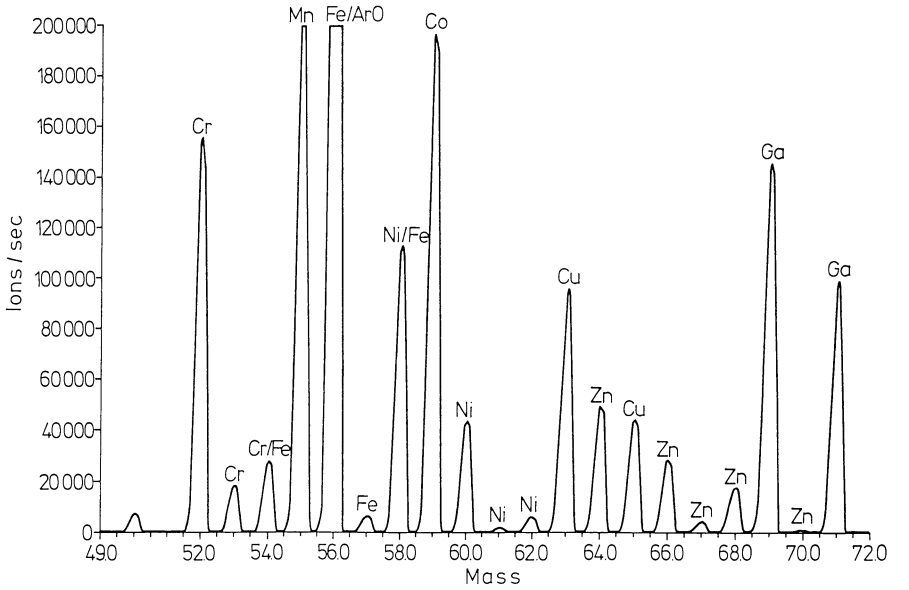

Fig. 10.4. Typical ICP-MS spectrum in the mass range 49–72 au. (Völlkopf and Paul 1989)

Table 10.3. ICP-MS analysis of a digested and 1:50 dilated kraft black liquor sample (μg/l)

Na	Mg	Al	Si	P	S	Cl	K	Ca
154000	1040	100	50	0.8	160	ND[a]	15400	500
Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn
1.2	5.0	27	140	43	0.1	0.5	40	45
As	Rb	Sr	Mo	Ag	Cd	Sn	Ba	Pb
0.2	56	3.3	1.2	0.2	0.3	1.5	16	3.6

[a] Not determined. Concentration is too high for the method.

For analysis of solid samples, a laser ablation (LA) unit can be coupled to the ICP-MS instrument. In modern LA instruments, a UV laser with wavelength of 266 nm is focused onto the spot to be analyzed. The laser beam evaporates a part of the sample and the sample vapors are directed with an argon stream to the ICP-MS instrument where the detection is done in the normal way. The size of the laser beam can be focused from 5 mm down to 10 μm. The detection limits of the LA-ICP-MS method for the elements in solid matrices are at the level of ppb (μg/kg). The detection limit strongly depends on the sample matrix. The LA-ICP-MS technique can be used, e.g., to screen the surface of a solid sample by moving the beam over the surface to be analyzed. At each spot, a complete mass spectrum is obtained. The depth profile of a

sample can also be determined by continuous "shooting" with the laser at one spot. With each "shot", the beam goes deeper and deeper into the sample and a complete mass spectrum of the vapors evaporated from the different depths of the sample can be obtained after each shot. The LA part of the instrument is also shown in Fig. 10.3.

10.3.3 X-Ray Fluorescence Spectroscopy

X-ray fluorescence spectroscopy (XRFS) is a well established analytical technique for quantitative determination of major and minor inorganic constituents in solid samples such as wood, chips, bark, pulp, furnace melt, dust, lime, lime mud, grit, dregs, etc. (Keitaanniemi and Virkola 1978). The XRFS method is a nondestructive method where the solid sample is irradiated with X-rays. The atoms absorb the primary X-ray radiation and are transferred into an excited state. When they relax to the ground state, a fluorescence X-ray radiation characteristic of each element is emitted. Three basic types of X-ray fluorescence instruments are commercially available, namely wavelength dispersive, energy dispersive, and nondispersive. Certain radioisotopes have also been used as the radiation source, especially in portable equipment intended for environmental and geochemical monitoring.

Sample preparation for an XRF measurement is an important step. The sample has to be dried, ground (preferably to a particle size finer than 200 mesh), homogenized, and pressed into pellets. Because the XRF technique allows only measurement of the surface of the sample, the signal is sensitive to the particle size of the sample prepared. Samples can also be made by fusion with borates or some other reagents. Liquid samples with a thickness exceeding 5 mm can also be analyzed with the XRFS technique.

The quantitative analysis is based on measurement of the net intensity (counts): the peak signal minus the background signal of a characteristic line of the element to be determined. Due to the relative simplicity of the emitted X-ray spectrum, only a few cases of spectral interference occur. The XRFS method is rather sensitive to the matrix, and therefore analytical errors caused by matrix effects easily occur due to absorption and/or enhancement of the radiation. The detection limit of XRFS for direct analysis of solid samples is in the order of 10–100 ppm (mg/kg), depending on the element. XRFS is a good method for multielement analysis of solid samples. The size of the irradiated area of the sample is in the order of 5 mm^2, and therefore the method cannot be used for analysis of small spot sizes.

In the electron microprobe method, the excitation is effected with a narrow beam of electrons focused on the surface of the specimen. Electromagnetic lenses are used in focusing the beam. The diameter of the beam is between 0.1 and 1 μm. A microscope can be used to locate the spot to be analyzed. In detection of the X-ray fluorescence emission, either wavelength or energy dis-

persive instrumentation is used. The electron microprobe X-ray analyzer-scanning microscope technique has been used to study mineral contents in selected cellular tissues (Wardell and Hart 1973).

Bombardment of specimens by protons can also give fluorescent X-rays. In particle induced X-ray emission (PIXE), protons of several million electron volts (MeV) of energy from an accelerator are used for excitation of atoms in the sample. The detection limit for PIXE analyses for most elements is in the range of 1–10 ppm (mg/kg). PIXE is a typical multielement method and less matrix-sensitive than XRF. The drawback of PIXE is that the technique can be used only in combination with an accelerator.

10.3.4 Electron Spectroscopy

The electron spectroscopic techniques are mainly used to characterize surfaces. Of all the contemporary surface analytical methods, ESCA is the most widely used. X-ray photon spectroscopy (XPS) is another name for the same method. ESCA is a high vacuum technique, where the sample is placed in a vacuum of at least 10^{-5} torr. The sample is then irradiated with photons in the X-ray energy range. The emission signal is a beam of electrons, the kinetic energy of which is measured. The low-resolution ESCA spectrum is used for qualitative elemental analysis. The technique is capable of detecting all the elements in the periodic table except hydrogen and helium, if the elements are present at concentrations >0.1 atomic %. The high-resolution spectrum can be used for determination of the oxidation state of an element and also its molecular environment, i.e., the type of bonds it forms. The method is also able to reveal the different bonds of carbon atoms. ESCA can be used both in nondestructive and destructive modes. Nondestructive measurements are done in the outermost 10 nm of the surface. The spot size of the irradiation beam is oval, with dimensions of approximately 1.0 mm $\times$ 1.5 mm. The destructive mode can be used for determination of elemental depth profiles of several hundred nanometers by using ion etching of the surface. Fingerprinting of materials using valence band spectra and identification of bonding orbitals is also possible with ESCA.

Auger electron spectroscopy is another surface analytical technique and similar to ESCA. The instrumentation used in both methods is also similar. In the Auger technique, the excitation of the sample is done with a beam of electrons, and the signal is the kinetic energy of the emitted electrons as in the ESCA.

A low resolution ESCA spectrum of pine pulp, sampled before bleaching, is shown in Fig. 10.5. The major constituents, carbon and oxygen, can easily be seen in the spectrum. Some sodium and silicon are also present. The major sodium peak is given by the Auger technique. A high resolution ESCA spectrum of the same pulp sample is shown in Fig. 10.6. Three peaks can be

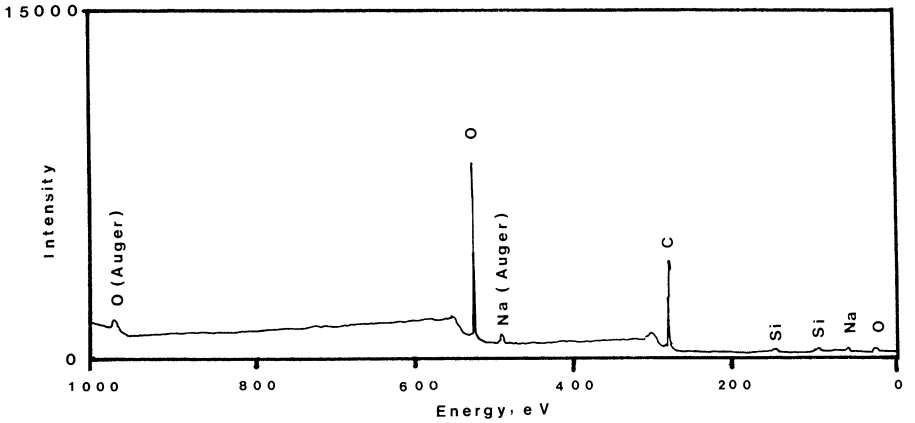

Fig. 10.5. Low resolution ESCA spectrum of pine pulp before the bleaching step

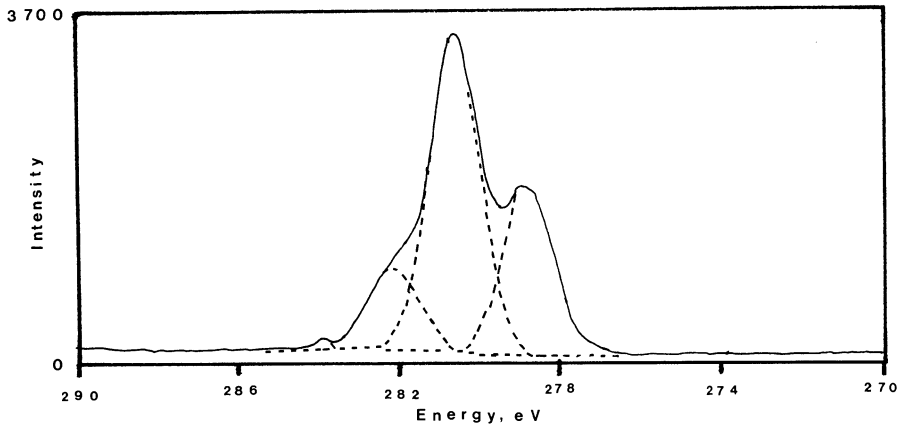

Fig. 10.6. High resolution ESCA spectrum of pine pulp before the bleaching step

seen in the spectrum. The peak to the right originates from hydrocarbons, indicating that the sample does not entirely consists of carbohydrates. Obviously some extractive substances have been adsorbed on the surface of the fibers. The peak in the middle originates from carbon, probably in carbohydrates, bound to one oxygen atom, and the peak to the left is from carbon bound to two oxygen atoms. There is a tiny shoulder on the high energy part of the left peak, which represents a carbonyl carbon, indicating the presence of carboxylic groups.

10.4 Ion Chromatography

Chromatographic methods are used to separate closely related components in complex mixtures. Detection after the separation of ions is done often with a detector that is not selective to individual species but sensitive to a group of compounds. Separation of inorganic components by ion chromatographic (IC) methods is based on the ion exchange equilibria between the ionic groups in the stationary phase and the ions in the sample solution.

IC was developed in the mid-1970s from ion exchange chromatography. The IC technique rapidly became an important method for determination of anions like fluoride, chloride, bromide, nitrite, nitrate, phosphate, and sulfate. IC can also be used for determination of inorganic cations, but those ions are normally determined by other methods.

The stationary phases in anion IC are anion exchange resins containing either quaternary amine groups $-N(CH_3)_3^+HO^-$ (strong base) or primary amine groups $-NH_3^+HO^-$ (weak base). When the sample containing anions is injected in the eluent stream flowing through the column, the anions will be attached to the ion exchange groups in the stationary phase. Different anions will be attached with different strength, depending on the equilibrium constant of the particular ion exchange reaction. As a result, the anions will pass through the column at different rates. The eluate in anion chromatography is normally a solution of sodium carbonate/hydrogen carbonate. To suppress the background conductivity of the eluant, the flow from the separation column is led to a suppressor column. The packing in a suppressor column is the acid form of a cation exchange resin. The cations of the eluate solution are exchanged to form carbon dioxide. The conductivity of the eluant from the suppressor column results mainly from the anions of the sample. A flow-through conductivity detector is the most common detector used in IC.

It is also possible to separate anions without the suppressor column. In this approach, the small differences in conductivity between the anions in the sample and the ions in the eluant solution are amplified. The ion exchange resin used has a low capacity (0.020 meq/g resin) and small particle size (5–10 μm). It is possible to use aqueous solutions of phthalic, benzoic, or salicylic acids in low concentrations as eluants.

The qualitative analysis is based on the retention time of the different ions. In quantitative analysis, either the peak height or the peak area is measured. Sample solutions should first be filtered, for example through a 0.45-μm membrane, before they are injected into the cloumn. This procedure improves the practical separation capacity of the column and increases its lifetime.

Ferretti et al. (1992) have determined P, Cl, and S in wood samples by combining the Schöniger combustion method with the subsequent determination of the ions formed by IC.

10.5 Miscellaneous Methods

10.5.1 Activation Analysis

Neutron activation analysis (NAA) is the most common form of the activation analytical methods. Thermal neutrons produced in a nuclear reactor are used to irradiate the sample and to create induced radioactivity in the sample atom. When a neutron is captured by the nucleus of an atom, it is transformed into a labile form having high energy. This excess energy is then released mainly by gamma-ray emission. The intensity of the radiation is a measure of the number of the target atoms in the sample, and the energy of radiation is element specific. The NAA is a sensitive method; the detection limit for many elements is in the range of 0.001–10 μg. Most of the elements in the periodic table can be determined by this technique.

NAA is a nondestructive method, and insensitive to the matrix. Its main disadvantage is that a nuclear reactor is required. The time of analysis is also long. Irradiation of the sample is normally from several minutes to hours.

Young and Guinn (1966) have used NAA for determination of several elements in wood and bark samples of different trees. They have also studied the distribution of the elements in the trees.

10.5.2 Secondary Ion Mass Spectrometry

Secondary ion mass spectrometry (SIMS) is a highly sophisticated surface analysis technique. The sample surface is bombarded with a beam of high energy positive ions. Ar^+ is the main ion used. The impact of the primary ions strip off the atoms from the surface. A small part of the stripped atoms are ionized, forming a beam of secondary ions which is led into a mass spectrometer. The diameter of the primary ion beam is in the range of 0.3 to 5 mm. SIMS is a sensitive technique capable of detecting amounts down to 10^{-9} μg. Concentration profiles with a depth resolution of 100 Å can be determined.

10.6 Concluding Remarks

Wood and pulp represent a typical biological matrix where the inorganic components are minor constituents. Process streams, however, represent a complex matrix containing organic (fibers) and inorganic particles and high concentrations of dissolved chemicals, most of them added in large quantities during the manufacturing processes. Sodium is present in high concentrations, as can be seen in Table 10.3, and causes interference due to its ionization

Table 10.4. Detection limits of some atomic spectroscopic methods (ppb or μg/l). (Anonymous 1997)

Element	Flame AAS	GFAAS	ICP-AES	ICP-MS
Ag	1.5	0.02	0.9	0.003
Al	45	0.1	3	0.006
As	150	0.2	50	0.006
Au	9	0.15	8	0.001
B	1000	20	0.8	0.09
Ba	15	0.35	0.09	0.002
Be	1.5	0.008	0.08	0.01
Bi	30	0.25	30	0.0005
Br				0.2
Ca	1.5	0.01	0.02	0.05
Cd	0.8	0.008	1	0.003
Ce			5	0.0004
Cl				10
Co	9	0.15	1	0.0009
Cr	3	0.03	2	0.02
Cs	15			0.0005
Cu	1.5	0.1	0.4	0.003
Dy	50		2	0.001
Er	60		1	0.0008
Eu	30		0.2	0.0007
F				10000
Fe	5	0.1	2	0.005
Ga	75		4	0.001
Gd	1800		0.9	0.002
Ge	300		20	0.003
Hf	300		4	0.0006
Hg	300	0.6	1	0.004
Ho	60		0.4	<0.0005
I				0.008
In	30		9	<0.0005
Ir	900	3.0	5	0.0006
K	3	0.008	20	0.015
La	3000		1	0.0005
Li	0.8	0.06	0.3	0.0001
Lu	1000		0.2	<0.0005
Mg	0.15	0.004	0.07	0.007
Mn	1.5	0.035	0.4	0.002
Mo	45	0.08	3	0.003
Na	0.3	0.02	3	0.003
Nb	1500		10	0.0009
Nd	1500		2	0.002
Ni	6	0.3	5	0.005
Os	120		6	
P	75000	130	30	0.3
Pb	15	0.06	10	0.001
Pd	30	0.8	3	0.003
Pr	7500		2	<0.0005
Pt	60	2.0	10	0.002
Rb	3	0.03	30	0.003

Table 10.4. *Continued*

Element	Flame AAS	GFAAS	ICP-AES	ICP-MS
Re	750		5	0.0006
Rh	6		5	0.0008
Ru	100	1.0	6	0.002
S			30	70
Sb	45	0.15	10	0.001
Sc	30		0.2	0.02
Se	100	0.3	50	0.06
Si	90	1.0	3	0.7
Sm	3000		2	0.001
Sn	150	0.2	60	0.002
Sr	3	0.025	0.03	0.0008
Ta	1500		10	0.0006
Tb	900		2	<0.0005
Te	30	0.4	10	0.01
Th				<0.0005
Ti	75	0.35	0.4	0.006
Tl	15	0.15	30	0.0005
Tm	15		0.6	<0.0005
U	15000		15	<0.0005
V	60	0.1	0.5	0.002
W	1500		8	0.001
Y	75		0.3	0.0009
Yb	8		0.3	0.001
Zn	1.5	0.1	1	0.003
Zr	450		0.7	0.004

effect, especially when atomic spectroscopic methods are used. Different analytical methods show great differences in their matrix sensitivities. NAA and PIXE are the least matrix sensitive of the methods described in this chapter.

The validity of an analytical method to be used is most conveniently assessed by using certified reference materials (CRMs) with a matrix composition similar to that of the sample. Some CRMs for wood are commercially available: NIST pine needles (National Institute of Science and Technology), CBR beech leaves (Community Bureau of Reference), and Swedish biofuel standards (Swedish University of Agricultural Sciences). Sometimes parallel methods are used for quality control of the analytical method used.

AAS has been the dominating wet-chemistry method in analytical laboratories for about three decades. Today it is often replaced by ICP-AES and DCP-AES which offer several advantages over AAS. The use of ICP-MS will certainly increase in the future, due to the high sensitivity of the method and its capability for multielement analysis. Sensitivity is an important factor when choosing an analytical method for a specific assay. Table 10.4 shows the detection

limits for different elements with different atomic spectroscopic techniques (Anonymous 1997). The best detection limits are attained using ICP-MS and graphite furnace AAS.

When analyzing inorganic constituents in wood, pulp, and related materials, many other factors except sensitivity affect the choice of the analytical method. The method to be used in a particular analysis depends greatly on elements or species to be determined in the sample. The equipment and instrumentation available also affects the choice of the method. Reliability and accuracy of the determination are also important parameters to be considered when choosing the determination technique. The fast development of analytical instrumentation and methods makes the work of an analyst easier, but, on the other hand, makes the selection of the proper method more difficult. So far, there is no universal analytical method that can be used for determination of all inorganic components; normally a number of different techniques are required to obtain reliable analytical data.

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