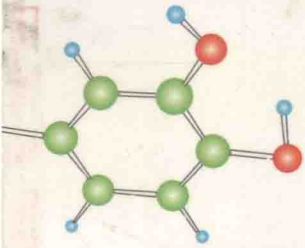

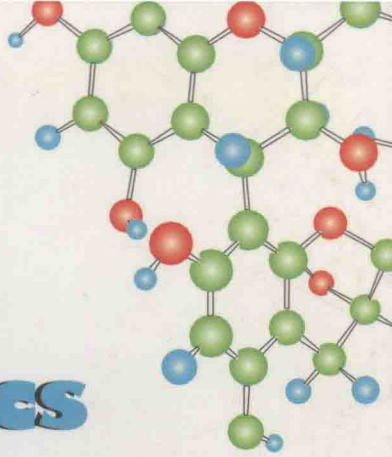

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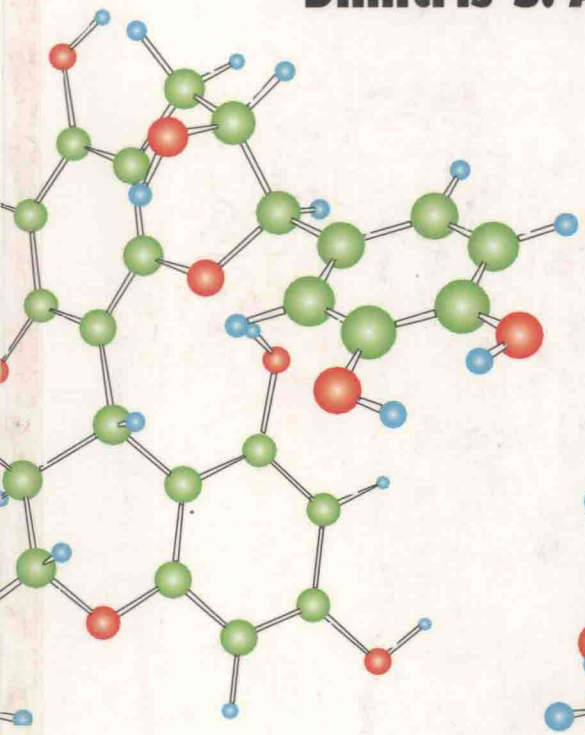

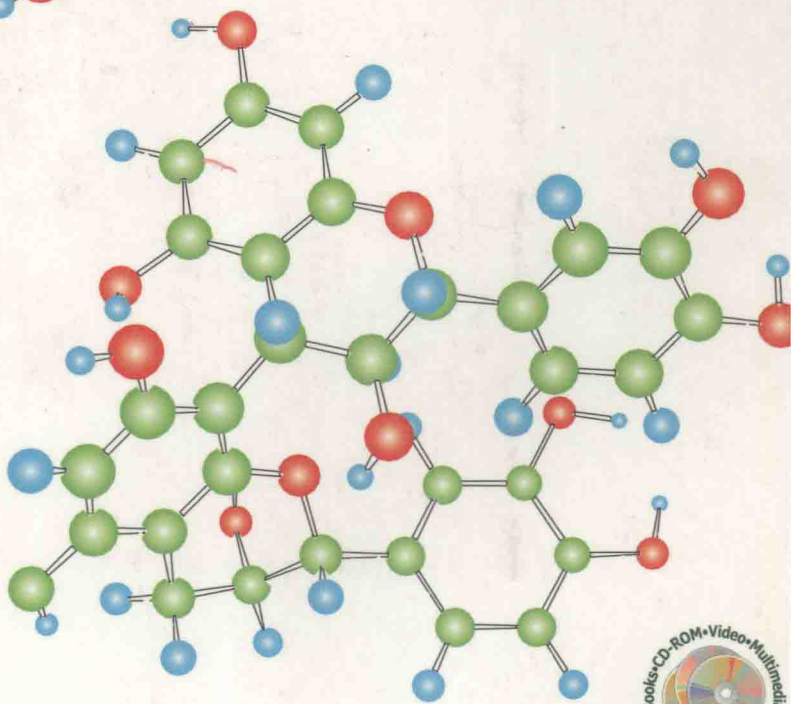

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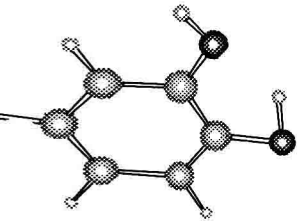

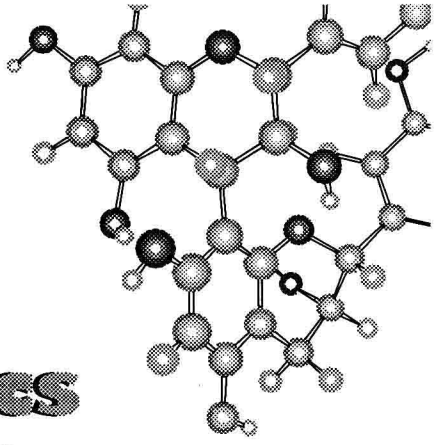

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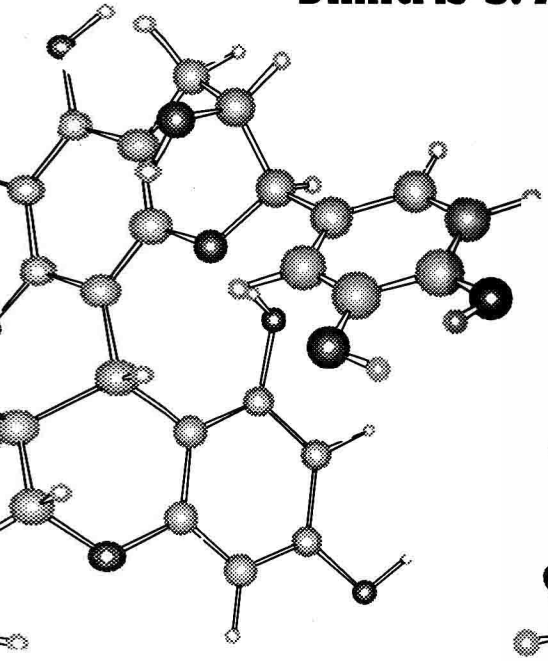



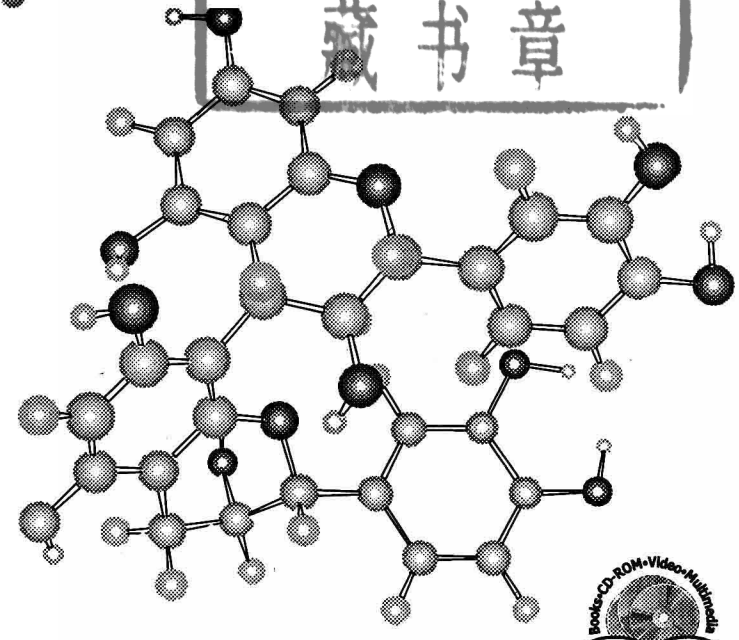

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# PREFACE

As we approach the new millennium issues of sustainability and environmental responsibility are becoming of increasing industrial importance. In this context our industry is well positioned since wood is a renewable resource. The manufacturing processes of paper and related products, despite their considerable complexity, are amenable to developments that promote an ecological balance with nature. Any process alterations, however, almost invariably imply significant structural changes that accompany property variations that may seriously impact the quality and eventually the marketability of our products. Proper understanding of structure-property relationships is, therefore, of paramount importance as we strive to develop manufacturing processes that may conform to the stringent regulatory and economic forces of the new millennium.

In November 1997, a symposium that was held in Cancun, Mexico, entitled "Structure and Properties of Lignocellulosic Materials" brought together a number of prominent researchers that disseminated the latest developments in the field. It was soon realized that a dominant message was about to emerge from this meeting focused on considerable advances in the area of lignocellulosics characterization. Advances in instrumentation coupled with novel ways to probe the structure of lignocellulosic substrates were described. Spectroscopic, chromatographic and microscopic techniques applied on lignin, on cellulose on paper and wood were shown to offer new levels of structural awareness. This valuable information is now compiled in *Advances in Lignocellulosics Characterization.*

The book is divided into various thematic sections that describe advances in the structure of wood components, Magnetic Resonance, Infra-red, UV and Raman spectroscopies as well as chromatographic, microscopic and computational techniques. Despite the fact that this book emerged from a scientific meeting it is not a collection of papers solely documenting specific findings in the form of conference proceedings. Instead, a collection of authoritative reviews is provided prior to embarking on the specific developments. An additional feature of this book is the use of color in selected figures providing exceptional clarity to the complex information that is derived from various spectroscopic techniques. It is anticipated that this book will offer valuable guidance to the endeavors of researchers in industry and academia, ensuring their awareness to the latest *Advances in Lignocellulosics Characterization.*

This book became possible due to the concerted effort of many individuals. The enthusiastic contributions of Dr. Tim Rials of the Unites States U.S. Forest Service, Pineville, LA toward coorganizing the aforementioned symposium are gratefully

acknowledged. The monetary contribution of the Cellulose, Paper and Textiles Division (CELL) of the American Chemical Society made the attendance of selected speakers possible and deserves our gratitude. No book of this nature could be produced without the support of the international scientific community. In this context the efforts of thirty nine scientists, dispersed throughout the globe, in reviewing the manuscripts were an invaluable contribution that deserves a grateful acknowledgment. These individuals ensured that the material in each chapter is of high scientific standard, reflects the state-of-the-art and is presented in the best possible way. Last but not least, the countless contributions of Mrs. Susanne Chatterjee in providing secretarial editorial assistance made this book possible.

Dimitris S. Argyropoulos
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August 1998

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# Chapter 1

# Isolation and Characterization of Residual Lignin

*Tarja L. Tamminen and Bo R. Hortling*

## INTRODUCTION

The aim of chemical pulp manufacturing, both pulping and bleaching, is to induce lignin dissolution without harmful effects on pulp polysaccharides. For lignin to be rendered soluble, it should be depolymerized, hydrophilic groups should be introduced, and lignin-carbohydrate bonds should be broken. To investigate the reactions of pulp lignin during the different treatments, lignin must be isolated from the pulp matrix and analyzed.

Analysis of pulp residual lignin gives valuable information about the structure of that proportion of the lignin that was unreactive during the previous delignification stage. The type of reactive sites in lignin may also suggest which delignification agents would be most suitable for that particular type of lignin. Determining the structure of residual lignin has been a challenge to research groups for many years.

The first critical step in residual lignin analysis is to isolate lignin from the pulp matrix without causing structural changes. Secondly, the analysis itself is complicated by the heterogeneous nature of lignin. The third aspect that should be kept in mind is the heterogeneous nature of the pulp fibre. The chemical composition of the cell wall layers varies even in the natural state, and also after delignification. Another type of heterogeneity can be found between individual fibres. This may be due to natural variation (summer versus spring wood fibres, reaction wood versus normal wood) or to technical factors such as mixing. This means that even if the perfect lignin isolation procedure could be found, the product would at best represent the average structure of the residual lignin components. Even so, information obtained about the structure of residual lignin is always valuable.

In the following, a general overview is given of the isolation and analytical procedures available for residual lignin. A more detailed description is provided in this chapter for selected methods, which have been developed and used at KCL, together with examples of the results obtained.

# METHODS USED IN RESIDUAL LIGNIN RESEARCH

## Isolation methods

Although many chemical, mechanical and enzymatic methods have been developed for the isolation of lignin from wood and pulp fibres, no method is available for the quantitative isolation of native and residual lignin without the risk of structural changes during the isolation. The two most widely used methods are described below.

### Acid hydrolysis

Residual lignin may be isolated from pulp chemically by extraction with acidic dioxane. This method has been described in detail for birch kraft pulps *(1)*. Removal of extractives is necessary prior to residual lignin isolation, especially with birch pulps. The extracted pulp is then hydrolysed under nitrogen with 0.1 M HCl in dioxane:water (82:18) under reflux (88°C). The solubilized lignin is recovered from the solution. Some of the residual lignin remains in the pulp, as shown by kappa number determination. The yield of residual lignin does not correspond to the calculated amount based on kappa number, being substantially lower. This is at least partly due to the existence of hexenuronic acid groups in the pulps, which contribute to kappa number *(2)*. The actual yield of lignin is thus better than indicated by the calculation. The residual lignin obtained is free from carbohydrates and other impurities. On the other hand, the acid treatment may change the lignin structure. Cleavage of the remaining $\alpha$-aryl and $\alpha$-alkyl ether linkages together with $\beta$-aryl ethers in benzyl alcohol structures is believed to take place, but no condensation reactions are probable *(1)*. Some structural changes have been suggested to take place in hardwood and softwood pulps due to the acidic conditions *(3)*.

### Enzymatic hydrolysis

A method based on total enzymatic hydrolysis of pulp for the isolation of residual lignin was first presented by Yamasaki *(4)*. By employing commercial cellulase enzymes, residual lignin is obtained in good yield, especially from unbleached kraft pulps. It was suggested that the small amounts of carbohydrates represent the polysaccharides that were originally linked to the residual lignin in the pulps.

The enzymatic method has been modified and applied to the analysis of residual lignin during bleaching *(5)*. To isolate lignin from oxygen delignified pulps, the method was modified by shortening the incubation time, but the repeated treatment technique was retained in order to obtain moderate lignin yields, but divided into several fractions *(6)*. Solvent swelling of the pulp sample has been found to enhance enzymatic digestability *(7)*.

Another modification using a single 48-hour enzyme treatment has been described *(8)*. Another cellulase product is used in this procedure, together with β-glucosidase, both of which are effective enough to hydrolyse practically all pulp polysaccharides during one incubation. The detailed procedure of this method, using commercially available enzymes, is presented later in this chapter.

The samples obtained by the enzymatic method contain protein impurities originating from the enzymes used in the hydrolysis step. The samples obtained need to be purified before analysis to remove most of the protein impurities. Chemical methods like alkali extraction/acid precipitation *(9)* or solvent extraction in several steps with dioxane/water *(10)* and DMAc (11) have been used. Protease purification has been used to remove most of the impurity, and this method is described in detail later in this chapter.

Compared to the isolation of residual lignins by acidic extraction, enzymatic methods have some benefits. For one thing, the residual lignin obtained can be considered to be chemically unchanged and the yield is quite good, especially for kraft pulps (>80%). However, carbohydrate and protein contaminants complicate analysis of lignin structure.

## Analytical methods

A detailed review of lignin chemistry was published in 1992 *(12)*, containing monographs about the isolation and characterization of residual lignin. The following is therefore restricted largely to the most recent literature.

### Spectroscopic methods

#### FTIR spectroscopy

One of the most widely used spectroscopic methods for lignin analysis is FTIR spectroscopy. The routine FTIR method for lignin analysis employs the mid-infrared region and the KBr pellet technique.

The band assignments have been investigated, and published data can be used for interpretation of the FTIR spectra of unknown lignin samples *(13)*.The bands are partly overlapping, but some structural features like aromatic structures, carbon-oxygen bonds and carbonyl groups are clearly detectable, and the different types of carbonyl groups can be assigned separately. Some methods for the semiquantitative determination of carboxylic acids, including certain other carbonyl structures, have been proposed *(14,15)*.

More information can be obtained by derivatization, *e.g.* by acetylation, which enhances the detectability of certain functionalities. This way, the ratio between phenolic and aliphatic hydroxyl groups can be calculated based on the two acetoxy bands at slightly different wavelengths *(16,17,18)*.

For native lignins like milled wood lignin, the information obtained from the FTIR spectrum includes indication of its botanical origin based on the contents of guaiacyl, syringyl and *p*-hydroxyphenyl type structures *(19)*. Analysis of pulp residual lignins is, however, complicated by the structural changes occurring during the delignification processes, and thus the methods of natural lignin analysis must be applied with caution to residual lignin analysis.

## UV spectroscopy

Ultraviolet spectroscopy is another traditional method that is still useful in lignin analysis. Because of its aromatic structure, lignin can easily be detected in the UV region. On the other hand, the UV spectra of different lignin samples are very similar due to overlapping bands, and structural information cannot be obtained. Thus, the most widely used application of UV spectroscopy is for the quantification of lignin in solution, or determination of the purity of an isolated sample. For both purposes, the absorptivity value for the specific type of lignin must be known.

Special UV techniques have been developed for the determination of functional groups. Ionization of phenolic hydroxyl groups in alkaline solution causes a bathochromic shift. This phenomenon can be used to determine the content of phenolic hydroxyl groups by measuring the ionization difference spectrum of the lignin sample. However, the overall structure of the lignin units carrying the phenolic hydroxyl groups affects the observed wavelengths and absorption intensities in the ionization difference spectra. This complicates the interpretation of the results, necessitating a separate calibration for each type of lignin, which is inconvenient for the analysis of unknown samples.

A correlation between the wavelength of the absorption maximum and the corresponding molar extinction coefficient has been determined, and this can be used to determine different types of phenolic structures separately *(20)*. Another method for taking into account the effect of the different lignin structures has been presented *(21)*. The advantage of this method is that the cross-effects between the absorption maxima are also taken into account by using the absorption at the two most informative wavelengths at the same time. This method, in slightly modified form, has been described for the determination of phenolic hydroxyl groups *(22)*. An application of the same method for the determination of the phenolic hydroxyl content in solutions is described later in this chapter.

## NMR spectroscopy

NMR spectroscopy is one of the most widely used methods for detailed structural characterization of lignin. It can be used for soluble derivatized and underivatized lignin samples. The signal assignments for both $^{1}H$ NMR *(23)* and $^{13}C$ NMR *(24,25,26)* have been published, and provide information about both the aromatic and side chain structures of lignin. Modern 2D NMR techniques provide even more structural information. The sensitivity of the $^{13}C$ NMR analysis of acetylated samples can be enhanced by using $^{13}C$-enriched acetyl chloride for the derivatization *(27)*. The $^{13}C$ NMR technique has been used to determine muconic acid type structures in oxidized lignins *(28)*.

More recently, solid state NMR has also been applied following the development of the Cross Polarization / Magic Angle Spinning (CP/MAS) technique, which improves resolution *(29)* and makes it possible to characterize partly or totally insoluble lignin samples. The information obtained in solution and solid state has been compared *(30)*.

Detection of nuclei other than $^{1}H$ and $^{13}C$ have proved to be useful for the determination of functional groups. $^{31}P$ NMR, in particular, has been widely used for the quantification of different types of hydroxyl groups in lignin *(31,32)*. New methods based on $^{19}F$ NMR spectra for derivatized lignin samples have been developed for the analysis of different types of carbonyl groups *(33,34)*.

## Wet chemical methods

In spite of the development and increased application of spectroscopic methods for lignin analysis, wet chemical methods are still used to obtain additional information. Derivatization combined with subsequent specific reactions is the basis for several methods developed for the determination of the functional groups in lignin.

Aminolysis is widely accepted as giving the most reliable content of phenolic hydroxyl groups. For this method, the sample is first acetylated and then reacted with pyrrolidine to induce deacetylation. The rate of deacetylation is faster for aromatic than aliphatic hydroxyl groups, a fact that is used for the quantification *(35)*. However, the method is tedious to perform.

Periodate oxidation is a straightforward method for quantification of phenolic hydroxyl groups. It is based on oxidation of the phenol to *o*-quinone with simultaneous liberation of methanol *(36)*. Only those phenolic structures carrying methoxyl groups in the *ortho*-position are quantified, which in most cases is not a serious restriction. Both aminolysis and periodate oxidation can be applied direct to wood or pulp meal *(37,38)*.

Methods for the determination of phenolic groups have recently been compared *(39,40)*. A round robin investigation has also been performed in which samples representing different types of lignin were analysed using several methods *(41)*. Of the methods used, aminolysis was considered to be the most reliable in both comparisons.

Like phenolic hydroxyl groups, carboxylic acid groups increase the hydrophilic nature of lignin. They are formed during oxidative delignification stages *via* side chain oxidation or aromatic ring cleavage leading to muconic acid structures. Potentiometric and conductometric titration methods are mainly used to determine the content of acidic groups in lignin. However, interpretation of the results is complicated because of the heterogeneous structure of lignin. Conductometric titration with a variety of bases has been performed using lignin model compounds *(42)*.

The total content of carbonyl groups can be determined by wet chemical methods after derivatization *(43,15)* or by spectroscopic methods *(39)*.

**Degradative methods**

An important approach in lignin analysis is to degrade the polymeric lignin molecule to monomeric or dimeric units, which are then identified. The structures and the relative or absolute contents of the degradation products are used as an indication of the original lignin structure.

Permanganate/hydrogen peroxide oxidation is a method which gives information about the substitution patterns of lignin and the frequency of linkages between lignin subunits. The sample is first alkylated, then oxidized and finally the acids formed as degradation products are esterified for the analysis *(44,45)*. A new modification of the permanganate oxidation method has been developed to prevent losses of volatile degradation products *(46)*. A drawback of the method is that only free phenolic lignin structures are detected unless the sample is pretreated to release more free phenolic lignin units.

Thioacidolysis gives information about the side chain structure of lignin, mainly the content of $\beta$-aryl ether structures. Lignin is reacted with ethanethiol in anhydrous medium with boron trifluoride etherate as catalyst *(47,48,49)*. Side reactions affecting the composition of the reaction mixture are minimal, and this method gives reliable data, especially for native lignins.

A new modification of thioacidolysis, the DFRC (Derivatization Followed by Reductive Cleavage) method, has recently been published *(50,51)*. Derivatization of lignin, accompanied by cell wall solubilization, is accomplished with acetyl bromide,

and the reductive cleavage of the resulting β-bromoethers is achieved with zinc in an acidic medium. Following acetylation, the degradation monomers are quantified by GC. The structural information is similar to that obtained by thioacidolysis, but the analysis is more convenient to perform.

Analytical pyrolysis of a lignin sample under well controlled conditions degrades it thermally into monomeric products. The product mixture can be led through a gas chromatograph into a detector. The products can be identified, most conveniently using mass spectrometric detection. The product spectrum is dependent on the polymeric structure of the lignin sample and can be used for lignin characterization in the same way as other degradative methods *(52)*. Detailed information about the identified degradation products has been published, which helps with interpretation of the analytical results *(53,54)*. Further improvements and modifications to the Py-GC/MS method have been suggested, like the use of an internal standard for better quantitation accuracy *(55)*, and *in situ* methylation of the lignin sample to yield further structural information *(56)*.

The analysis is easy to perform as no complicated sample pretreatment is needed. Non-phenolic as well as phenolic lignin units can be determined. The result is thus more representative of the whole sample and especially suitable for the analysis of pulp residual lignins. However, the correlation between the products observed and their origin is not yet well documented.

**Molar mass**

The molar mass of residual lignin is of great interest, as depolymerization is one of the important reactions leading to pulp delignification. Absolute molar mass values of lignins are difficult to determine due to structural heterogeneity and associations between lignin molecules *(57,58,59)* and also because the structures of the calibration standards differ from those of lignin. The structures of lignins vary from well-defined milled wood lignins to oxidized and condensed residual lignins and lignins present in spent bleaching liquors. It is possible to determine the molar masses of lignins either direct from the spent liquors or after precipitation at pH 3 from the spent liquors. However, precipitated lignins represent only a part of the lignin in the spent liquor due to the solubility of low molar mass and hydrophilic lignin fractions. The possible association of lignin molecules during the precipitation must also be accounted for.

Gel permeation chromatography has mostly been performed in aqueous alkaline systems using Sephadex gels and a UV detector measuring at 280nm. The molar masses of lignins from pulping and bleaching spent liquors have been determined using this method *(57,58,60,)*. Absolute molar mass values are difficult to determine due to the apparent difficulty of finding monodisperse lignin calibration standards. It is possible, however, by using proteins and Na-polystyrene sulfonate standards to

calculate relative molar masses. It is thus possible to follow changes in the molar masses of lignins with similar structures reliably by using constant GPC conditions. Semirigid gels like TSK Fractogel HW-55F (Merck), which are based on a porous, synthetic hydrophilic resin, and Superdex gels, which are based on highly cross-linked porous agarose beads to which dextran has been covalently bonded, have been used in GPC measurements using aqueous alkaline media *(8,61,62)*. These gels allow GPC runs to be performed fairly quickly, yielding results that are similar to those obtained by Sephadex gels.

In order to avoid association phenomena and to perform analyses in a shorter time, HP/SEC (High Performance/Size Exclusion Chromatography) analysis using rigid divinylpolystyrene gels and organic eluents like THF is used. Because of the low solubility of most lignins in organic solvents, the lignin first has to be derivatized. Several applications of this method are mentioned in a comprehensive work on the characterization of different lignin samples *(63,64)*.

Generally UV and/or RI detectors are used for the monitoring of lignins in GPC and HP/SEC. However, it is also possible to perform universal calibration using viscosity detectors *(63,65)* and light scattering detectors. Most lignins are soluble in DMAC/0.8% LiCl, which is of special interest because the same eluent is used to determine the molar mass distribution of both unbleached and semibleached pulps. It is thus possible to compare the molar mass distribution of residual lignin in the pulp and after isolation from the same pulp. Such investigations clearly indicate that the molar mass of the isolated residual lignin is much lower than when measured *in situ* in the pulp, supporting the statements that residual lignin is actually linked to polysaccharides in the pulp.

**Lignin-carbohydrate bonds**

The chemical and/or physical attachment of residual lignin to pulp polysaccharides is another factor that affects the dissolution properties of the lignin. Much work has been done to study the occurrence and type of lignin-carbohydrate bonds in wood materials. Native lignins have been isolated by both chemical and enzymatic methods *(66,67,68)*. It has generally been stated that enzymatically isolated lignin represents the total native lignin better than mechanically and chemically isolated lignin. In addition, enzymatically isolated lignins provide information about the interactions between lignin and carbohydrates.

The aim is to understand the formation of lignin and lignin-carbohydrate complexes during the growth of the cell wall *(69,70,71,72,73)* and in this way to obtain information for the development of delignification reactions during pulping and bleaching reactions *(74)*. These studies report that enzymatically isolated native lignin