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RESONANCE RAMAN SPECTROSCOPY IN THE ANALYSIS OF RESIDUAL LIGNIN AND OTHER UNSATURATED STRUCTURES IN CHEMICAL PULPS

Anna-Maija Saariaho

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Department Department of Forest Products Technology
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Opponent(s) Professor Göran Gellerstedt
Supervisor Professor Tapani Vuorinen
(Instructor) Docent Anna-Stiina Jääskeläinen

Abstract

Ultraviolet resonance Raman spectroscopy (UVRRS) was applied to determine the characteristic vibrations of several monomeric and dimeric lignin model compounds. The characteristic vibrations of p-hydroxyphenyl, guaiacyl, and syringyl lignin structures were then detected in the UVRR spectra of extracted wood samples. Further, a partial least squares (PLS) model was developed to assist the determination of the characteristic UVRR bands of different lignin structures. The UVRRS-PLS method was further applied to interpret the more complex lignin spectra. The results correlated well with the results obtained by Py-GC/FID for the quantification of guaiacyl and syringyl lignin structures and with results obtained by NMR spectroscopy for the quantification of condensed lignin structures in guaiacyl-type lignin.

Chromophoric lignin structures were studied by exciting pulp samples in the visible range. The overwhelming fluorescence emission was suppressed by applying a carbon disulfide (CS2) based Kerr gate technique. The fluorescence rejection ratio was estimated to be as high as 250. Chromophores in chlorine dioxide and peroxide bleached pulps were of different nature, as indicated by the different correlations with brightness and kappa number.

Residual lignin and hexenuronic acids in the unbleached and bleached chemical pulps were quantified with PLS calibration and with direct band height ratios. PLS calibration gave relatively good predictions, while the direct band height ratio was more a straightforward method. The band height ratio method is not, however, applicable to unbleached pulps with lignin contents of about 5% because in these pulps the cellulose bands are indiscernible and cannot be used as a reference band.

Carbonyl groups in cellulosic pulps were studied by the UVRRS and by the CCOA (carbazole-9-carbonyloxyamine) method, which showed that part of the carbonyls exist in hydrate, hemiacetal and/or hemiketal forms. It was also shown that these structures interconvert upon drying and wetting of the pulps. UVRRS was efficient in detecting extremely low contents of carbonyls, unlike IR spectroscopy, which did not detect any differences in the carbonyl band of these pulps.

Keywords carbonyls, hexenuronic acid, Kerr gate, lignin, model compounds, resonance Raman spectroscopy

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PREFACE

Most of the work described in this thesis was carried out in the Laboratory of Forest Products Chemistry at the Helsinki University of Technology during the years 2001-2004. Part was carried out in the Central Laser Facilities at the Rutherford Appleton Laboratory, UK, in 2002.

I wish to express my deepest thanks to Professor Tapani Vuorinen for his ideas, inspiration, and assistance throughout my work. Not only did he help me in several practical ways, but he also spent considerable time reading my manuscripts. He set the framework for the work, which otherwise would have continued endlessly. I am further grateful to my instructor, Docent Anna-Stiina Jääskeläinen, for her guidance and support. I profited from many fruitful discussions and she always found time to listen and assist. Dr. Pavel Matousek, Dr. Mike Towrie, Dr. Anthony W. Parker, and Dr. Stanley Botchway are thanked for their help at the Rutherford Appleton Laboratory, where I was introduced to the fascinating world of molecular behavior at picosecond scale. Mrs. Rita Hatakka provided skillful practical assistance, and the staff at the Laboratory of Forest Products Chemistry created a great working atmosphere.

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Espoo, 13th May 2004
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following six articles, which are referred to in the text by their Roman numerals (I-VI). Some new results are also included.

I Saariaho, A.-M., Jääskeläinen, A.-S., Nuopponen, M., Vuorinen, T., Ultra violet resonance Raman spectroscopy in lignin analysis: Determination of characteristic vibrational bands of *p*-hydroxyphenyl, guaiacyl and syringyl lignin structures, Applied Spectroscopy, 57:1 (2003) 58-66.

II Saariaho, A.-M., Hortling, B., Jääskeläinen, A.-S., Tamminen, T., Vuorinen, T., Simultaneous quantification of residual lignin and hexenuronic acid from chemical pulps with UV resonance Raman spectroscopy and multivariate calibration, Journal of Pulp and Paper Science, 29:11 (2003) 363-370.

III Saariaho, A.-M., Jääskeläinen, A.-S., Matousek, P., Towrie, M., Parker, A.W., Vuorinen, T., Resonance Raman spectroscopy of highly fluorescing lignin containing chemical pulps: Suppression of fluorescence with an optical Kerr gate, Holzforschung, 58:1 (2004) 82-90.

IV Saariaho, A.-M., Argyropoulos, D.S., Jääskeläinen, A.-S., Vuorinen, T., Development of the partial least squares models for the interpretation of the UV resonance Raman spectra of lignin model compounds, accepted to Vibrational Spectroscopy.

V Jääskeläinen, A.-S., Saariaho, A.-M., Vuorinen, T., Quantification of lignin and hexenuronic acids in bleached hardwood kraft pulps: A new method based on UVRR spectroscopy and evaluation of the conventional methods, submitted to Journal of Wood Chemistry and Technology.

VI Potthast, A., Rosenau, T., Kosma, P., Saariaho, A.-M., Vuorinen, T., Sixta, H., On the nature of carbonyl groups in cellulosic pulps, accepted to Cellulose (2005).

The author's contribution to the appended joint publications:

I Anna-Maija Saariaho defined the research plan together with the co-authors, was responsible for the experimental work related to model compounds, analyzed the results, and wrote the manuscript.

II, IV Anna-Maija Saariaho defined the research plan together with the co-authors, was responsible for the experimental work, analyzed the results, and wrote the manuscript.

III Anna-Maija Saariaho defined the research plan together with the co-authors, performed the Kerr gate related experimental work, was responsible for the UVRR studies, analyzed the results, and wrote most of the manuscript. Dr. Pavel Matousek wrote the Kerr gate related parts in the introduction and the experimental sections of the manuscript.

V Anna-Maija Saariaho defined the research plan together with the co-authors, analyzed the results, and revised the manuscript. The original manuscript was written by Dr. Anna-Stiina Jääskeläinen.

VI Anna-Maija Saariaho defined the research plan together with the co-authors, was responsible for the experimental work related to UVRR studies, analyzed the corresponding results, and wrote the UVRR-related parts of the manuscript.

ABBREVIATIONS

CCOA	Carbazole-9-carbonyloxyamine
CK	Conventional kraft pulp
CPMAS	cross polarization/magic angle spinning
D	Chlorine dioxide bleaching
E	Alkaline extraction
FID	Flame ionization detector
FT	Fourier transform
FT	Flow through (in chapter 4)
FTIR	Fourier transform infrared
G	Guaiacyl
GPC	Gel permeation chromatography
H	*p*-Hydroxyphenyl
HexA	Hexenuronic acid
IR	Infrared
MWL	Milled wood lignin
NIR	Near infrared
NMR	Nuclear magnetic resonance
O	Oxygen delignification
P	Peroxide bleaching
PLS	Partial least squares
Py-GC/FID	Pyrolysis-gas chromatography-flame ionization detector
Py-GC/MS	Pyrolysis-gas chromatography-mass spectrometry
Py-MS	Pyrolysis-mass spectrometry
Q	Chelation
RL	Residual lignin
RRS	Resonance Raman spectroscopy
S	Syringyl
SB	SuperBatch

SEP	Standard error of prediction
SERDS	Shifted excitation Raman difference spectroscopy
SLL	Spent liquor lignin
UV	Ultraviolet
UVRR	Ultraviolet resonance Raman
UVRRS	Ultraviolet resonance Raman spectroscopy
Vis-RRS	Visible resonance Raman spectroscopy

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1 INTRODUCTION

In the manufacturing of chemical papermaking pulps, the wood chips are usually treated with alkaline sulfide in a process called kraft pulping. The wood fibers are liberated and large amounts of lignin are depolymerized and solubilized into the cooking liquor. The product is a brownish pulp with a lignin content of about 2-5%. The brightness of unbleached kraft pulp is of the order of 20-30% ISO units. The low brightness is mainly due to certain residual lignin structures that are more intensively colored than the natural lignin in wood. (Alén 2000, pp. 62-102)

The brightness of unbleached pulp is increased in subsequent bleaching processes. Higher brightness is obtained either by removal of the residual lignin or by chemical conversion of chromophoric structures. The first approach is the usual one for chemical pulps, and the lignin content in fully bleached pulp is about 0.1% and the brightness 90% ISO units. The bleaching is performed with chemicals such as oxygen (O), chlorine dioxide (D), peroxide (P), and ozone (Z).

The chemical analysis of lignin in both pulp and spent liquors during pulping and bleaching is of great importance, for several reasons. Information can be obtained on the chemical reactions taking place at different stages and the effectiveness of different bleaching chemicals can be evaluated. Moreover, knowledge of structures and amounts of lignin in pulp can be applied in the planning of further bleaching processes.

The structure of residual lignin is still not fully understood. It is clear, however, that the residual lignin is of rather high molecular weight (Sjöholm *et al.* 1999) and it is chemically bound to cell wall polysaccharides (Azuma *et al.* 1981; Isogai *et al.* 1987). Conventional wet chemical methods for the characterization of lignin continue to be of great importance, but increasingly they are being replaced by instrumental techniques that are less tedious and time consuming. The most common of these techniques are Fourier transform infrared (FTIR) spectroscopy (Faix 1992), nuclear magnetic resonance (NMR) spectroscopy (Leary

and Newman 1992), and pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) (Meier and Faix 1992).

Most of the analytical techniques require isolation of lignin or pretreatment of the wood or pulp samples. Isolation is difficult even from unbleached pulps (Chang 1992), and practically impossible from fully bleached pulps because of the low content and relatively high molecular weight of lignin and the chemical bonds between lignin and carbohydrates. The isolation step is always time consuming and laborious. Moreover, the naturally existing chemical bonds in lignin and between lignin and the cell wall polysaccharides are partly affected or destroyed during the isolation and thus the structure of lignin is altered. For these reasons, methods for characterization of lignin *in situ* are essential.

Lignin and some other pulp components can be characterized *in situ* by resonance Raman spectroscopy because of the high sensitivity and selectivity. These characteristics are obtained through resonance enhanced Raman signals of chemical structures that absorb at the excitation wavelength. Aromatic lignin and other unsaturated structures in chemical pulps are resonantly enhanced when the excitation wavelength is selected in the ultraviolet (UV) region. Additionally, chromophoric lignin structures are enhanced with visible excitation. Other pulp components, specifically polysaccharides, do not absorb radiation in either of these spectral regions, and their Raman signals are not enhanced. However, polysaccharides contain trace amounts of unsaturated structures, such as hexenuronic acids and carbonyl groups, which contribute to the resonance enhanced Raman scattering. For these reasons, low contents of absorbing structures can be analyzed directly, and characterization of lignin in fully bleached pulps without prior isolation is possible.

In this work, the concept “resonance Raman spectroscopy” is commonly used to refer to the method in which wavelengths permitting resonance enhancement are used. Even with these excitation wavelengths, saturated structures, such as celluloses, are not resonantly enhanced and their spectra are normal Raman spectra. For simplicity, all spectra, whether they are

resonantly enhanced or conventional Raman spectra, are regarded as resonance Raman spectra because of the used excitation wavelength.

2 AIMS OF THE STUDY

The main objective of this study was to evaluate ultraviolet resonance Raman spectroscopy (UVRRS) as a method of analyzing chemical pulps for residual lignin and other unsaturated structures. The research on this new technique was begun with model compound studies to assist interpretation of the lignin UVRR spectra (I). Model compound studies were redirected to partial least squares (PLS) modeling as it became evident that visual elucidation of the spectra would be too difficult (IV). Modeling was further used to assess the contents of selected lignin structures in isolated lignin samples, but this approach was hindered by the lack of reliable reference methods. It was also of interest to obtain resonance Raman spectra of lignins and pulps in the visible range. The aim here was to obtain information on residual chromophoric lignin structures as well as to show that the Kerr gated resonance Raman technique is directly applicable to solid lignin and pulp samples and capable of reducing disturbing fluorescence emission (III).

Besides the structural characterization of residual lignin, the quantitative nature of the UVRR data was exploited for quantifying lignin and hexenuronic acids in chemical pulps and carbonyls in cellulosic pulps. The objective of the quantitative study was to evaluate the different ways in which the residual lignin and hexenuronic acid contents of various pulps can be determined. PLS calibration was demonstrated with softwood pulps (II) and the direct band height ratio method with hardwood pulps (V). The aim of the carbonyl study was to characterize the minor carbonyl groups in cellulosic pulps, which are not detected by infrared (IR) spectroscopy. This was done by comparing the results obtained by UVRRS and by the CCOA (carbazole-9-carbonyloxyamine) method which is used to quantify carbonyls (VI).

3 ANALYTICAL METHODS FOR ANALYZING LIGNIN

This chapter reviews the analytical methods for studying lignin. Section 3.1 on the structural characterization of lignin focuses on spectroscopic techniques, while section 3.2 on the quantification of lignin in pulps describes, in addition, some of the conventional wet chemical methods.

3.1 STRUCTURAL CHARACTERIZATION OF LIGNIN

Most of the analytical and spectroscopic methods require isolation of lignin prior to analysis. Isolation alters the chemical structure of polymeric lignin, however, and it is always a laborious and time consuming step. The most common isolated lignin from wood is milled wood lignin (MWL) (Björkman 1956), which is considered to represent wood lignin. The isolation of lignin from pulps is generally accomplished by enzymatic (Pew 1957; Yamasaki *et al.* 1981) or acid hydrolysis (Gellerstedt *et al.* 1994) or their combination (Argyropoulos *et al.* 2000), in which the pulp carbohydrates dissolve and lignin precipitates. The isolation of lignin from bleached pulp is more difficult because of the low concentration and rather high molecular weight of lignin (Sjöholm *et al.* 1999) and its chemical linkages with carbohydrates (Azuma *et al.* 1981; Isogai *et al.* 1987). Direct analytical methods are necessary when studying the structural details of residual lignin in bleached pulps. Until recently, isolation has been seen as a necessary step in the characterization of residual lignin even in unbleached pulps (Al-Dajani and Gellerstedt 2002). Several techniques by which the chemical structure of lignins can be studied, directly or as isolated lignin, are discussed below. The emphasis is on spectroscopic techniques.

FTIR spectroscopy

Fourier transform infrared (FTIR) spectroscopy, which gives information on vibrational energy levels of the sample molecule, is widely used in lignin characterization (Faix 1992). IR spectroscopy in general is sensitive towards polar bonds and information on aromatic

lignin structures is not easily obtained. FTIR has been used to investigate several features of differently isolated lignins, including monomer type (*p*-hydroxyphenyl, guaiacyl, syringyl) (Reis Machado *et al.* 1996; Martínez *et al.* 1999; Seca *et al.* 2000), methoxyl groups, carbonyl groups (Hortling *et al.* 1997; Faix *et al.* 1998; Seca *et al.* 2000), and phenolic (Wegener and Strobel 1991; Faix *et al.* 1992; Faix and Böttcher 1993; Faix *et al.* 1994; Ramos *et al.* 1999; Gilarranz *et al.* 2001) and aliphatic hydroxyl groups (Faix *et al.* 1994).

IR techniques are sensitive to moisture and only dried samples can be analyzed. This is a major drawback of the method and an obstacle to the implementation of FTIR in process control, for example. So far, FTIR has been used only for isolated lignins and the direct analysis of lignin structures in pulps is not possible. FTIR spectroscopy is widely used, however, and its capabilities are well-known. It is easily adapted for numerous analytical purposes, therefore.

NMR spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy provides information on sample atoms and different environments can be distinguished. Modern high-resolution NMR techniques are even capable of distinguishing different morphologies of the sample, for example the amorphous and crystalline cellulose (Liitiä 2002).

NMR analysis can be performed in either solid state or solution. NMR analysis of solutions and liquids gives sharp bands because all chemical interactions within the sample are averaged (Sjöholm 2001). Modern techniques, most notably cross polarization/magic angle spinning (CPMAS) NMR, also provide high resolution spectra for solid samples, a development that has facilitated the characterization of lignin (Leary and Newman 1992). Solid state NMR analysis of wood and related materials has been extensively reviewed by Gil and Neto (1999). Conventional and modern multidimensional NMR techniques for solutions have widely been used for the characterization of various lignin structures (Lundquist 1992; Robert 1992), but because they involve the additional pretreatment step

of dissolution, they can be considered more laborious. Furthermore, some lignin structures are difficult to dissolve entirely. A recent review discusses in detail the use of NMR spectroscopy in analyzing wood and its products (Maunu 2002). As there are so many studies and techniques related to NMR spectroscopy, only solid state CPMAS NMR is discussed here. This technique can be seen as a competing technique to UVRR spectroscopy since it, too, provides information on solid lignin samples.

The resolution of CPMAS NMR spectroscopy of solid lignin samples can be enhanced by several special techniques, including dipolar dephasing. The use of these special techniques has allowed the determination of the syringyl to guaiacyl ratio in extracted hardwoods (Manders 1987), estimation of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) in hardwood lignins (Hawkes *et al.* 1993), the degree of substitution of the aromatic ring in lignin (Hatcher 1987), and the degree of condensation of guaiacyl-type lignin (Liitiä *et al.* 2000, 2001, 2002). CPMAS alone, without an enhancing technique, has also been used to analyze the syringyl to guaiacyl ratio of hardwood lignins (Hatfield *et al.* 1987; Martínez *et al.* 1999). Phosphorus-31 (^{31}P) NMR deserves mention for the possibility it allows of analyzing mechanical pulps directly for chromophoric lignin structures such as *o*-quinone groups (Argyropoulos *et al.* 1992; Argyropoulos 1995).

The major drawback of all NMR techniques is the data acquisition time which may run from several hours to days. This is in comparison with a few minutes in UVRR spectroscopy. Moreover, solid state NMR spectroscopy has only been utilized for isolated lignins, extracted wood and chemically treated pulps; the direct analysis of lignin in pulps is impossible with existing NMR techniques.

Raman spectroscopy

Raman scattering is produced if the polarizability of a chemical bond is changed during an inelastic collision with incident radiation. Raman scattering is most intense from nonpolar bonds with symmetrical charge distributions, unlike IR, which detects mostly polar bonds.

Raman spectroscopy also gives information on the vibrational energy levels of the sample constituents.

The first Raman spectroscopic studies on lignocellulosic materials concentrated on the orientation of lignin and cellulose in wood cell walls (Atalla and Agarwal 1985, 1986; Agarwal and Atalla 1986). Extensive work in the interpretation of Raman spectra of lignins has been done by Ehrhardt, Atalla, Agarwal, and their co-workers (Ehrhardt 1984; Atalla 1987; Agarwal *et al.* 1997; Agarwal and Ralph 1997). Assignments of lignin Raman bands are mainly based on their work.

The use of near-IR (NIR) FT Raman in analyzing lignocellulosic materials increased in the 1990s (Kenton and Rubinovitz 1990; Evans 1991; Weinstock *et al.* 1993a, 1993b; Takei *et al.* 1995; Agarwal *et al.* 1997; Ibrahim *et al.* 1997; Sun *et al.* 1997). The technique offers fluorescence-free or fluorescence-suppressed Raman spectra and enables fast and simple data acquisition (Agarwal *et al.* 1997). NIR excitation does not, however, allow the use of resonance enhancement due to the lack of electronic transitions in this spectral region. All pulp components contribute equally to the spectra and direct characterization of residual lignin in pulps is difficult, and from fully bleached pulps impossible. NIR FT Raman has been used to study certain lignin characteristics, such as α,β-unsaturated bonds (Kihara *et al.* 2002), carbonyl groups (Kihara *et al.* 2002), and syringyl and guaiacyl groups (Takayama *et al.* 1997; Ona *et al.* 1998). Resonance Raman spectroscopy in the visible range has been applied to study chromophores (Agarwal *et al.* 1995; Agarwal and Atalla 2000) with use of molecular oxygen to suppress the fluorescence.

UV resonance Raman spectroscopy offers information on the chemical structure of residual lignin directly, even from fully bleached pulps. This has not earlier been achieved with other spectroscopic techniques. UVRRS was first applied to the analysis of residual lignin in unbleached kraft pulp in 1990 (Sukhov *et al.* 1990). Shortly afterwards it was applied to lignin (Sukhov *et al.* 1993). Unfortunately, there was no follow-up, and the topic was buried for almost 10 years until Halttunen *et al.* (2001a) published a study on UVRRS of

residual lignin in bleached pulps. Thereafter, UVRR spectroscopy has been applied to the study of residual lignin in softwood pulps (Perander *et al.* 2001; Halttunen *et al.* 2001b), residual lignin in softwood and hardwood pulps (Jääskeläinen *et al.* 2003), and residual chromophores in bleached pulps (Mateo *et al.* 2002). It has also been applied to investigate the changes taking place in lignin structure during thermal modification of softwood (Nuopponen *et al.* 2004).

Agarwal and Atalla (1995) mentioned time-resolved gating system in the Raman apparatus, which they used in order to obtain the Raman spectrum of lignin-containing samples in the visible range without disturbing fluorescence emission. In view of the "overall complexity" of the system, they did not publish any results.

A drawback to the utilization of Raman techniques is the difficulty in changing the excitation wavelength. The selection of the wavelength is also usually limited to a few choices depending on the laser at hand. It needs to be added that Raman techniques are unfamiliar to many scientists. Instrumentation is relatively expensive, the cost varying, among other things, with the excitation wavelengths required.

Py-GC/MS and Py-GC/FID

Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) deserves mention as an important technique for characterizing polymeric samples. It is also widely used in the characterization of lignin (Meier and Faix 1992). The technique involves fragmentation of the sample in an inert gas at high temperature, after which the fragments are separated in a gas chromatographic column and identified with a mass spectrometer. The analysis can also be accomplished with a flame ionization detector (FID), or the fragments can be identified directly with a mass spectrometer without gas chromatographic separation. The analysis requires a minimal sample amount and it can be accomplished directly without an isolation procedure. The method is destructive, but only about 5-50 μg of sample is required. The technique allows the determination of *p*-hydroxyphenyl, guaiacyl, and syringyl structures

(Rodrigues *et al.* 1999; del Río *et al.* 2001; Sonoda *et al.* 2001) as well as some other characteristic structures of lignin polymer. Information on the chemical structure of residual lignin is obtainable directly from pulp samples.

3.2 QUANTIFICATION OF LIGNIN IN PULPS

3.2.1 WET CHEMICAL METHODS

The conventional wet chemical methods for quantification of lignin in pulps involve measurements of kappa number and of total lignin.

Kappa number

Kappa number is a common measure of lignin content in unbleached and semi-bleached pulps (Dence 1992). Since it describes the content of oxidizable structures with potassium permanganate (SCAN-C 1:00), other structures than lignin also contribute to the kappa number determination. Moreover, if the pulp has been delignified or bleached with oxidizing agents, part of the lignin structures will be oxidized already and the kappa number will be erroneously low. The greatest contribution of other oxidizable structures is from hexenuronic acid (HexA) groups that are introduced to the pulp during alkaline pulping (Teleman *et al.* 1995). This contribution must be subtracted from the kappa number in order to obtain a figure for the real lignin content (Li and Gellerstedt 1997). Several aspects need to be kept in mind, therefore, when drawing conclusions about lignin contents in pulps from the kappa number. Moreover, kappa number is not a reliable measure of the lignin content of bleached pulps with lignin content less than 2-5%. This is mainly because the residual lignin in bleached pulps is oxidized and consumes less permanganate than the natural lignin structures (Li *et al.* 2002).

Klason lignin and total lignin

The Klason lignin, which is acid-insoluble lignin, is determined by weighing the solid residue of extracted wood or unbleached pulp after its treatment with sulfuric acid, H_2SO_4 (Dence 1992). The acid-soluble lignin is determined from the filtrate by measuring the absorbance at 205 nm. The total lignin of wood and unbleached pulps is obtained as the sum of the acid-insoluble and soluble lignins. The method is not applicable to bleached pulps mainly because of the inaccuracy in weighing the insoluble lignin residue. Total lignin content is considered to be the best measure of lignin in wood and unbleached pulps (Brunow *et al.* 1999).

3.2.2 SPECTROSCOPIC METHODS

Ultraviolet

Lignin can be quantified in wood and pulps by UV spectrometry (Bolker and Somerville 1962). The method is based on measurement of the absorbance at 210 or 280 nm of a finely ground sample in a potassium chloride pellet. Lignin is quantified in a non-destructive way and the amount of sample required is minimal. The analysis is also relatively simple. The suitability of the method for quantifying lignin in hardwoods is questionable because of the different absorptivities of guaiacyl and syringyl lignins. Today, there are other spectroscopic techniques available that offer structural details simultaneously with quantitative data. These methods are discussed below.

FTIR

FTIR has been used for lignin determination, mainly in diffuse reflectance mode, but some shortcomings of the method are evident (Faix 1992). These include band overlapping and sensitivity to moisture. Moreover, all pulp components contribute to the spectrum, complicating the determination of lignin in bleached pulps.

A simple approach for the estimation of lignin in unbleached pulps is to determine the absorbance at 1510 cm^{-1} (Berben *et al.* 1987). The accuracy of the method is lowered by the overlap of the cellulose bands with the lignin band at 1510 cm^{-1}. The problem of band overlapping has been largely overcome by the multivariate approach. Utilizing a stepwise elimination regression, Schultz *et al.* (1985) obtained a relatively accurate prediction of lignin content with five absorbance variables. A similar approach was used by Grandmaison *et al.* (1987) and Schultz and Burns (1990). Backa and Brolin (1991) applied partial least squares (PLS) modeling, whereas Friese and Banerjee (1992) used a method based on minimizing the complexity of the spectra.

The advantage of FTIR spectroscopy is the relatively fast acquisition of the data and the simple pretreatment and measurement. FTIR spectrometers are widely available and well-known and their adaptation is relatively easy compared with the relatively unfamiliar Raman techniques.

Raman

NIR FT Raman has been used to quantify lignin from softwood (Weinstock *et al.* 1993b; Agarwal *et al.* 1996; Ibrahim *et al.* 1997; Agarwal *et al.* 2003) and hardwood pulps (Sun *et al.* 1997). NIR FT Raman does not suffer from fluorescence background, an obstacle in resonance Raman spectroscopy. This is because the excitation is performed at 1064 nm where no electronic transitions occur. The method offers a rapid and non-destructive way of determining lignin content in pulps. It is also insensitive to water and it holds a potential for at-line process control in pulping and bleaching mills (Ibrahim *et al.* 1997; Sun *et al.* 1997). These methods are not sensitive to low kappa numbers (*i.e.* ~ 4), however, a limitation that is stressed by Ibrahim *et al.* (1997) and Sun *et al.* (1997), and a reduced fluorescence background is required. Ona *et al.* (1997 and 2000) used second derivatives of NIR FT Raman spectra in PLS calibration and obtained good correlation with the kappa number and lignin content as well as with the some other pulp properties of eucalyptus pulp.

Halttunen *et al.* (2001a) demonstrated that the residual lignin in pulps can be quantified from the relative band intensities of lignin and cellulose measured by UV resonance Raman spectroscopy. Owing to the extremely high resonance enhancement of lignin, however, cellulose bands are indiscernible in the UVRR spectra of unbleached pulps and the results obtained are inaccurate. The method is applicable only to bleached pulps, and the measurements can be done *in situ*.

4 MATERIALS AND METHODS

Most of the samples studied in this work were described in papers I-VI. In addition to these, eleven other lignin samples were analyzed: 1) steam explosion lignin from yellow poplar (Round Robin), 2) organosolv lignin from mixed hardwoods (Round Robin), 3) sucrolin acid hydrolysis lignin from bagasse (Round Robin), 4) flow through spent liquor lignin after 60 minutes cooking (FT-SLL-60), 5) FT-SLL-120, 6) FT-SLL-240, 7) flow through residual lignin of pulp with kappa number 26.9 (FT-RL-26.9), 8) conventional kraft pulp residual lignin (CK-RL), 9) CK with oxygen delignification residual lignin (CK-O-RL), 10) CK with oxygen and peroxide bleaching (chelation prior to peroxide) residual lignin (CK-OQP-RL), and 11) SuperBatch residual lignin (SB-RL). The preparation of lignins 1-3 is described in detail by Milne *et al.* (1992). The lignins 4-7 and 11 are from pine (*Pinus radiata*) and lignins 8-10 from spruce (*Picea abies*). The preparation of lignins 4-10 is described in detail by Liitiä *et al.* (2002). Lignin 11 was isolated from SuperBatch pulp cooked at 170^{o}C after 185 min cooking time and had kappa number 11.1 (TEKES 1996). Pure cellulose was purchased from Whatman Inc. The UVRR results for these samples are discussed in sections 5.2 and 6.3.

The UVRR spectra of samples 1-11 were baseline corrected and the baseline was forced to zero. The spectra were scaled so that the intensity of the aromatic vibration band at about 1605 cm^{-1} was 10 arbitrary units. In this way, the variance in the spectra was the same as the variance in the model compound spectra (see details in paper IV) that were used for the construction of the PLS models (sections 6.1 and 6.3).

5 ADVANTAGES OF RESONANCE RAMAN TECHNIQUES

A unique feature of Raman spectroscopy is the possibility of selecting the excitation wavelength and gaining information on specific absorbing groups. It is also possible to obtain high sensitivity for selected structures by tuning the wavelength appropriately. Both selectivity and sensitivity are obtained through resonance enhanced Raman scattering of structures that absorb the excitation wavelength. Sensitivity can also be increased, in principle, by using lower excitation wavelengths because the Raman scattering is inversely proportional to the fourth power of the excitation wavelength. The resonance enhanced Raman intensities are up to 10^6 times as high as the conventional Raman intensities (Willard *et al.* 1988, pp. 321-325). Applied to pulps, resonance Raman spectroscopy (RRS) provides high sensitivity and selectivity toward residual lignin and other unsaturated structures. The analysis can be performed directly from pulp samples even where trace amounts are studied. The unsaturated structures absorb light in the visible and ultraviolet regions, generating resonantly enhanced Raman signals. Other pulp components, such as saturated carbohydrate structures, do not absorb light in these regions and their contribution to Raman scattering is negligible.

The utilization of RRS as an analytical tool has been hindered by an intensive fluorescence background. To induce the resonance enhancement, the sample needs to be excited with light that is close or equal in energy to the electronic transitions, and at these wavelengths, most samples start to fluoresce. Fluorescence emission is substantially more intense than Raman scattering and Raman signals are swamped under the fluorescence background. In pulp samples, the strong fluorescence emission is mainly caused by the residual lignin structures (Atalla *et al.* 1992). However, there are several publications, some dating back even to 1800s, about the fluorescence of celluloses as reviewed by Olmstead and Gray (1997). Basing on the review, it seems that the studies on fluorescence behavior of celluloses are affected by small impurities of lignin residues or some other unidentified minor components. In that review, it is concluded that the fluorescence emission originating from lignin is evident while that of celluloses remains ill defined (Olmstead and Gray

1997). It is reasonable to assume that the pure cellulose structure itself does not contribute to the fluorescence emission and that the observed fluorescence might have been due to some unsaturated impurities or minor unsaturated structures within the cellulose chain. This is because fluorescence requires an electronic transition to an excited electronic state and with UV and visible excitations these kinds of transitions are possible for unsaturated structures containing π-electrons, as far as organic molecules are concerned (Atkins 1994).

Fortunately, there are several ways in which the fluorescence problem can be overcome. The traditional techniques, which have also been applied to pulps, are drench quenching, water immersion, and oxygen flushing (Atalla and Agarwal 1986; Atalla *et al.* 1992; Agarwal 1999). All these techniques require long data acquisition times, and some chemical changes in the sample may occur. An alternative fluorescence suppression technique is essential, therefore, in analyzing lignin and pulp samples. These alternative techniques are excitation below the fluorescence emission in the UV region (Bowman and Spiro 1980; Li and Stair 1997; Sands *et al.* 1998; Halttunen *et al.* 2001a), shifted excitation Raman difference spectroscopy (Shreve *et al.* 1992) (SERDS), polarization modulation (Angel *et al.* 1984), shifted spectra (Mosier-Boss *et al.* 1995; Bell *et al.* 1998), Fourier transform filtering (Mosier-Boss *et al.* 1995), and temporal gating (Yaney 1972; Harris *et al.* 1976; Deffontaine *et al.* 1985; Howard *et al.* 1986; Fujii *et al.* 1988; Tahara and Hamaguchi 1993; Matousek *et al.* 1999, 2001; Everall *et al.* 2001). Excitation in the deep UV and temporal gating were applied to the lignin and pulp samples in this work.

5.1 DIFFERENT EXCITATION WAVELENGTHS (I & III)

The availability of different excitation wavelengths provides a means to obtain different kind of information from the sample constituents. Even two different excitation wavelengths within the UV region (229 and 257 nm) produced notably dissimilar resonance Raman scattering from some of the model compounds (I). The resonance Raman spectrum of 2-methoxy-4-methylphenol recorded with 229 nm excitation wavelength contained only one intense aromatic Raman band, at about 1600 cm^{-1}, whereas the spectrum recorded at

257 nm contained several strong and medium intensity bands in the 1500-700 cm^{-1} range. This indicated that 229 nm was more sensitive to aromatic structures but, detailed structural information remained unresolved. Sensitivity of the 229 nm wavelength to aromatic structures was evident, caused by the absorption band of the aromatic ring at 203 nm (Hase 1992). Hence, 229 nm would be an ideal wavelength for analysis of the low concentrations of aromatic lignin. The excitation at 257 nm, on the other hand, is more appropriate for the characterization of residual lignin structures. This finding was the main reason for the selection of wavelength 257 nm for the study of spectral features of lignin substructures (see sections 6.1, 6.3 and papers I and IV). Wavelength 229 nm was not used for the quantitative studies of lignin (see chapter 7 and papers II and V) because the output power of the laser was very much lower than that of the other available wavelengths and increased data acquisition times would have been required. Furthermore, the lignin concentrations in question were not extremely low, and higher excitation wavelengths were useful as well.

The resonance Raman spectra of conventional kraft cooking pulps with peroxide bleaching recorded with UV (257 nm) and visible (400 nm) excitations were also compared. The ratio of the intensities of the aromatic lignin band and the cellulose band was about 20 times higher with UV excitation than with visible excitation. Again, this was an indication that the lower excitation wavelengths are more sensitive towards aromatic structures.

5.2 UVRRS

Infrared excitation leads to non-enhanced Raman scattering and most of the chemical components in the sample contribute equally to the spectrum. Excitation in the visible range leads to resonance enhanced Raman scattering of absorbing groups, but the absorption of the radiation also produces fluorescence emission. When UV excitation is applied, the Raman scattering appears at shorter wavelengths than the fluorescence and fluorescence-free spectra are recorded.

UV excitation produces resonantly enhanced Raman signals of aromatic lignin and other unsaturated structures in pulps while the saturated pulp components, such as cellulose, contribute to the spectrum in non-enhanced mode. This Raman scattering arising from cellulose is low in intensity when compared to resonance Raman scattering of unsaturated structures. Wood extractives are also enhanced (Nuopponen *et al.* 2002), but these are dissolved in the early stages of pulping and do not contribute to the Raman spectra of pulps. High sensitivity and selectivity are thus obtained. The selectivity towards lignin structures is illustrated in Figure 1 in which the UVRR spectra of cellulose, fully bleached pulp, unbleached pulp, and residual lignin are shown. Only bands originating from residual lignin are detected in the spectrum of unbleached pulp, and the contribution from the matrix components, *i.e.* carbohydrates, is undetectable. The spectrum of bleached pulp contains resonance enhanced Raman bands originating from lignin and non-enhanced Raman bands from cellulose. The lignin band is the most intense, even though the lignin content in this pulp is only 0.1% as calculated from the kappa number (κ=0.6).

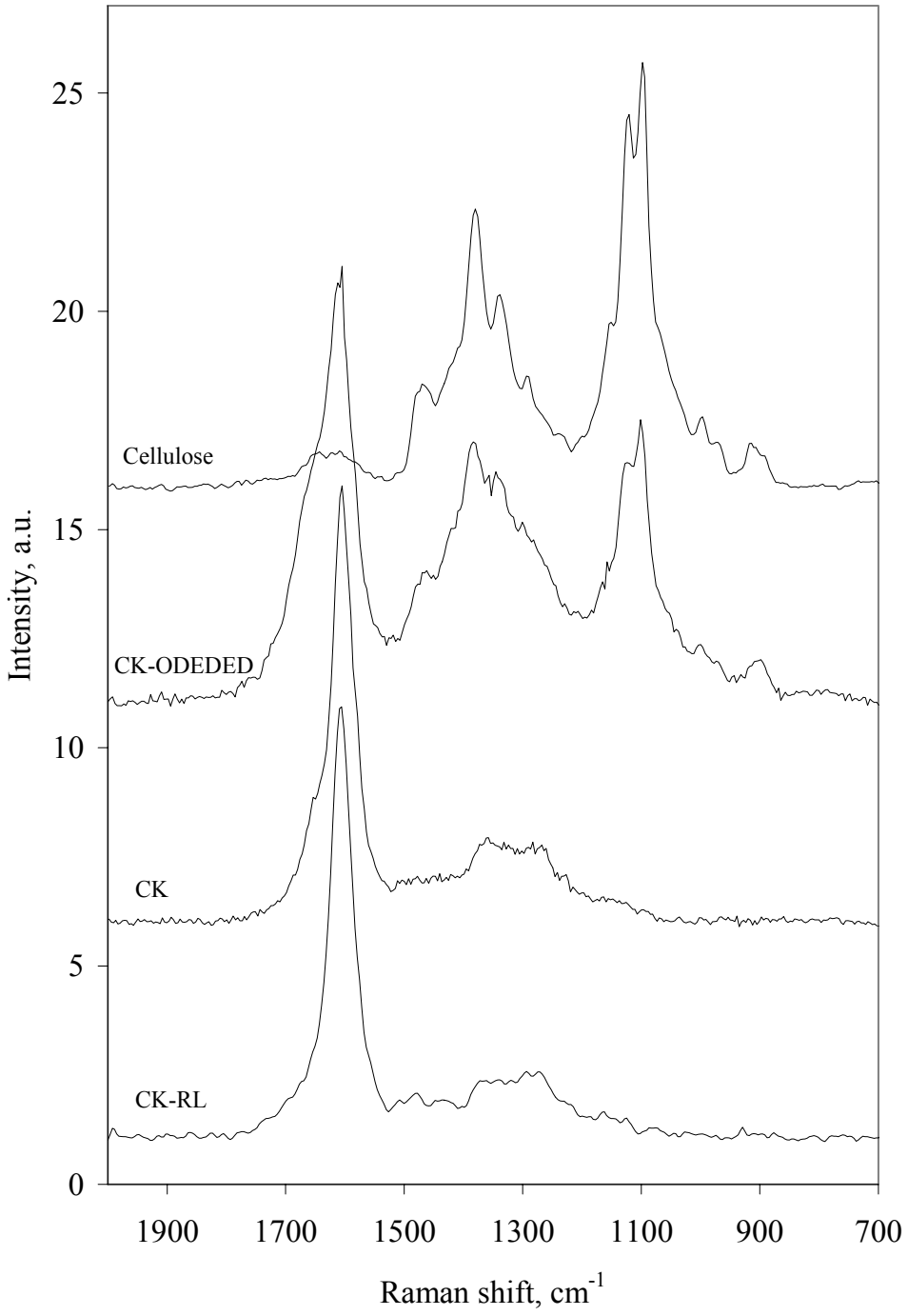

Figure 1. UVRR spectra of cellulose, fully bleached pulp (CK-ODEDED), unbleached pulp (CK), and residual lignin (CK-RL) recorded with 244 nm excitation.

5.3 Vis-RRS WITH KERR GATE SYSTEM (III)

The excitation in the visible region is of great importance for analytical purposes because of the high selectivity towards chromophoric structures. Unfortunately, visible excitation lies in the region where the fluorescence emission occurs and direct detection of the weaker Raman signals is prevented. A technique that suppresses the fluorescence is required for detection of the Raman signals.

The Kerr gate system (Matousek *et al.* 1999, 2001; Everall *et al.* 2001) is a direct fluorescence suppression technique and the spectra can be acquired in less than 10 min. The Kerr gate separates instantaneous Raman scattering and longer living fluorescence emission in the time domain: the gate is first in the open state, allowing the Raman scattering to pass to the detector, and then it is closed to prevent fluorescence emission from reaching the detector. This technique, *i.e.* temporal gating, was successfully applied, for the first time, to the analysis of pulps. The spectra of bleached conventional kraft cooking pulps showed intense fluorescence emission when Kerr gate rejection was not applied. The intense fluorescence emission, originating mainly from residual lignin structures (Atalla *et al.* 1992), prevented detection of the substantially weaker Raman signals. Fluorescence-free Raman spectra of pulps were collected with visible excitation (400 nm) by using a carbon disulfide (CS_2) based Kerr gate. The Kerr gate system was highly effective in suppressing the fluorescence emission; the rejection ratio was estimated at roughly 250.

6 STRUCTURAL CHARACTERISATION OF LIGNIN

6.1 SPECTRAL FEATURES OF LIGNIN SUBSTRUCTURES (I & IV)

Characteristic vibrations of the three different monomer types (*p*-hydroxyphenyl, guaiacyl and syringyl) of lignin polymer were first determined by visually comparing model compound spectra (I). Only the most prominent features could be identified. The information about characteristic frequencies enabled a rough, visual interpretation of Raman spectra. More detailed information on complex polymeric lignin samples is not easily available, however.

A more powerful method for interpreting the characteristic vibrations of lignin structures was accordingly sought. A PLS model was selected for the purpose because it had the potential for extension to a quantitative model (see section 6.3).

The PLS model was developed by quantifying the substructures of lignin model compounds (IV). In this way, the model was able to detect the characteristic Raman frequencies of each structure. The characteristic frequencies detected for the different monomer types were similar to those determined visually, but characteristic bands of low intensity were detected as well (see Table I). On the other hand, the loadings line spectra did not contain bands that were also characteristic of another model compound group/groups used in the models, so that the PLS model gave bands that were characteristic only to a particular structure. These results showed that the model is feasible and capable of detecting spectral features of model compounds.

Table I. Characteristic Raman frequencies of the three monomer types of lignin determined visually and with a PLS model.

	Visual determination (I)	PLS determination (IV)
p-Hydroxyphenyl		1488
		1405
	1390-1378	
		1338
	1297-1256	
	1217-1214	1215
	1179-1167	1164
		1094
	862-817	861-841
	644-637	644
Guaiacyl	1521-1517	1520
	1383-1372	
	1289-1285	1285-1270
	1274-1267	
	1187-1185	1186
	1158-1155	
		1124
		1078
		1024
	932-920	920
	791-704	784, 761
		711
Syringyl		1588
	1514-1506	1510
	1333-1330	1331
		1228
		1148
		1108
	981-962	964
	808-777	810
		741

The constructed PLS model was also used to determine characteristic vibrations of C5 condensed lignin structures, conjugated C=C and C=O structures, and stilbene structures.

The loadings line spectra of PLS models, which showed the characteristic Raman vibrations, were compared with the spectra of the corresponding model compounds. The most prominent bands of the loadings line spectra were clearly detected in the spectra of the model compounds, but the lower intensity vibrations were not detected. Some of the low intensity bands may be ghost bands that appear due to the frequency shifts of the different model compounds. Interpretation of the conjugated C=O structures was difficult because the appearance of the C=O stretching band is greatly altered by its chemical environment, which causes the band to appear in the relatively wide range of 1660-1700 cm^{-1}.

For the first time, PLS modeling was used in this way to interpret spectral data. The characteristic Raman vibrations of lignin substructures can further be utilized in the interpretation of the UVRR spectra of polymeric lignin samples, which typically contain broad and obscured signals. Such PLS models facilitate the interpretation of the highly complex UVRR spectra of polymeric lignin samples.

6.2 MONOMER TYPES OF LIGNIN IN EXTRACTED WOOD SAMPLES (I)

Visual elucidation of the Raman spectrum of extracted softwood revealed the existence of guaiacyl-type lignin. The spectrum contained three characteristic frequencies that were determined through visual comparison with the Raman spectra of monomeric lignin model compounds. The spectrum of hardwood contained, in addition, two Raman bands characteristic of syringyl lignin. Elucidation of the Raman bands of compression wood was more difficult because polymeric lignin in compression wood is a mixture of the three types of monomer and some of the visually determined characteristic vibrations are similar or close to each other.

Visual interpretation gives only a rough picture of the possible structures in the samples. Most other lignin samples, *e.g.* pulps and isolated residual lignins are more complex, and a similar interpretation of their Raman spectra may be impossible. Additionally, cellulose contributes to the Raman spectra of bleached pulp and complicates the structural

interpretation of the spectra. A visual approach is not useful, moreover, if quantitative data is required.

6.3 ABUNDANCE OF LIGNIN STRUCTURES IN LIGNIN SAMPLES (New data)

The PLS model that was developed (see section 6.1 and paper IV) can be used to estimate the abundance of different lignin structures. This approach is new in the characterization of polymeric samples. The models use the corresponding loadings line spectra to predict the content of a specific structure in polymeric lignin samples. The method requires calibration with another available method, *i.e.* a reference method. In fact, the major hindrance today in the utilization and development of such models is the lack of reliable and unambiguous reference methods. Additionally, many of the existing analytical methods give divergent results and it is difficult to say which are the true quantities of the different lignin structures. Anyhow, it was possible to compare some of the results with results obtained by other analytical methods. It needs to be kept in mind that the results obtained with the reference method may not represent the true contents. Moreover, there may be some contribution to the Raman results from overlapping bands, though the contribution should be relatively minor.

Monomer types

The UVRRS-PLS model was constructed with 11 monomeric model compounds (group A in paper IV: 4H, 4G and 3S) and 3 Y-variables (contents of H, G, and S structures as described in paper IV). The model explained 51.6% of the total variation in data with four principal components. The model was used to predict the guaiacyl and syringyl contents of three Round Robin lignin samples. Guaiacyl and syringyl contents of these same lignins have earlier been determined by pyrolysis-gas chromatography with flame ionization detector (Py-GC/FID) and by Py-mass spectrometer (Py-MS) with ammonia chemical ionization (van der Hage *et al.* 1993). The UVRRS-PLS results correlated significantly with those obtained by Py-GC/FID, whereas correlation with the Py-MS results was lower. The correlation coefficients are presented in Table II. It is worth noting that the correlation

between Py-GC/FID and Py-MS was lower than the correlation between UVRRS-PLS and Py-GC/FID. The correlation between UVRRS-PLS and Py-GC/FID can be seen in Figure 2. The better correlation of UVRRS-PLS results to Py-GC/FID than to Py-MS is most probably due to the fact that FID in general has wide linear range and its response is quite similar to most organic molecules, i.e. it is not specific to any chemical structure (Willard et al. 1988, pp. 552-560). Contrarily, the quantification of MS data is challenging because the response should be determined for all compounds separately with corresponding pure compounds (Willard et al. 1988, pp. 497-498).

Table II. The correlation coefficients between different methods to determine guaiacyl (G) and syringyl (S) contents in three Round Robin lignin samples. The Py-GC/FID and Py-MS results were published by van der Hage *et al.* (1993).

Correlation coefficient	Py-GC/FID	Py-MS
UVRRS-PLS	G: 0.9973 S: 0.9957	G: 0.8814 S: 0.8841
Py-MS	G: 0.9107 S: 0.9226	-

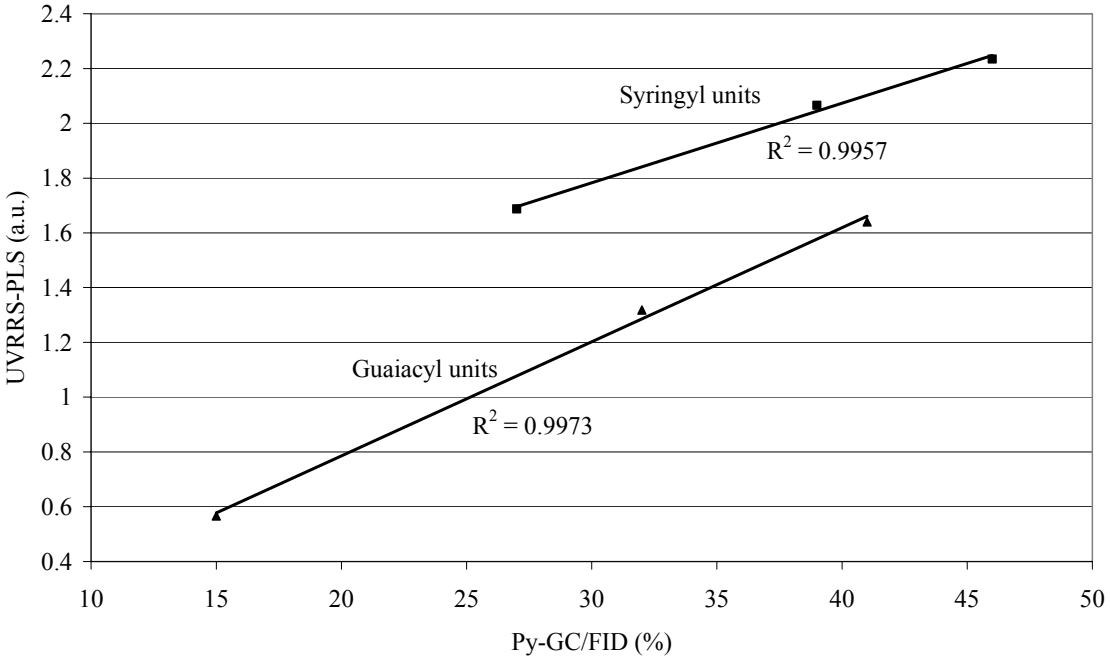

Figure 2. Correlation between UVRRS-PLS and Py-GC/FID results for guaiacyl and syringyl structures in Round Robin lignin samples. The Py-GC/FID results were published by van der Hage *et al.* (1993).

These results show that the PLS model is applicable and reasonable in predicting the monomer substitution pattern of lignin monomer units in polymeric lignin samples. Note that the results obtained directly from the model are presented in arbitrary units. The determination of real concentrations requires calibration of the arbitrary units against some reference method, such as Py-GC/FID. The real concentrations can be determined only from a calibration line. A different approach would be to use known polymeric lignin samples in the construction of the PLS model, in which, the results would be in real concentration units. This kind of approach is commonly used in multivariate data analysis and most probably it would work well here.

C5 condensed structures

A similar model was constructed to predict the content of C5 condensed lignin structures in polymeric lignin samples. The model was constructed with four guaiacyl-type monomeric and four C5 condensed dimeric model compounds with variable degrees of condensation (group B in paper IV) and two Y-variables (G and cond.). The model explained 54.3% of the total variation in data with only one principal component. The model was taught only to detect structures in which the condensation occurs at the C5 carbon in the guaiacyl units. The results are only valid, therefore, for softwood lignins where the C5 carbon is naturally unsubstituted and substituted only when condensed with other lignin or carbohydrate units during chemical pulping and bleaching. It may be noted that hardwood lignin is not as easily condensed as softwood lignin because it is characterized by a higher content of syringyl structures, which cannot be condensed at C5 carbon. The exclusion of hardwood lignins from this model is thus justified and reasonable.

The model was tested with eight softwood lignin samples that had been analyzed by CP/MAS NMR spectroscopy for their degree of condensation (Liitiä 2002, p. 33-34). The correlation coefficient was 0.884 and the correlation is illustrated in Figure 3. The result indicates that the UVRRS-PLS method can be used to predict the degree of condensation in softwood lignins.

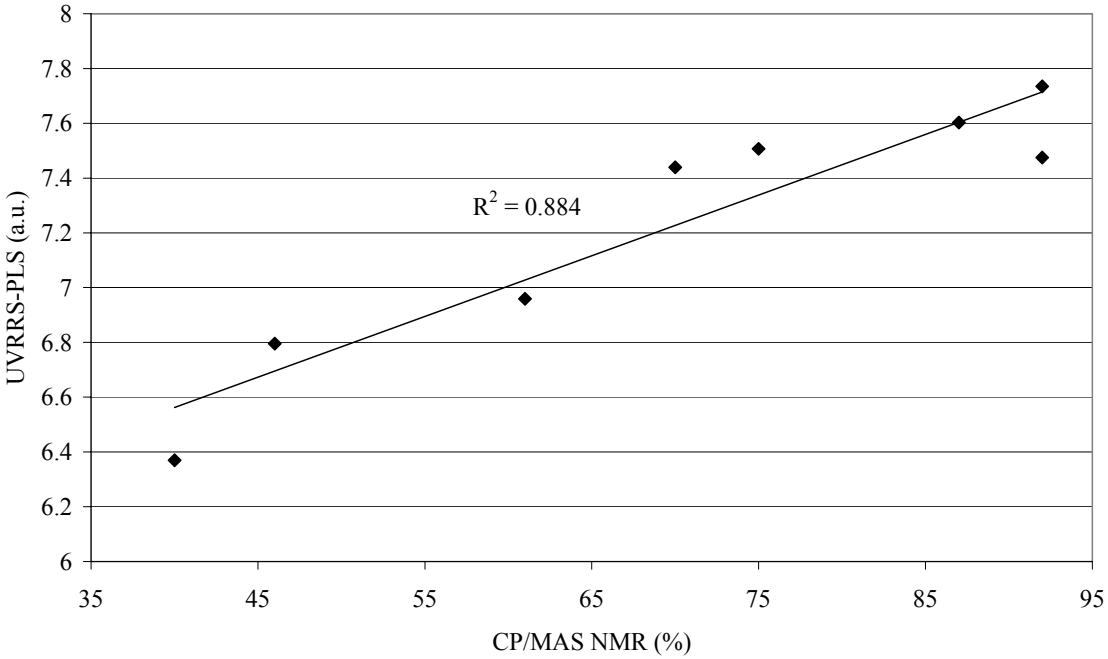

Figure 3. The correlation of C5 condensed structures of eight lignin samples determined by the UVRRS-PLS model and by CP/MAS NMR. The CP/MAS NMR results are from Liitiä (2002, p. 33-34).

6.4 NATURE OF CHROMOPHORES IN PULPS (III)

Chromophores absorb visible light and their electronic transitions occur when the samples are excited in the visible region. The chromophoric lignin structures were studied by vis-RR spectroscopy (400 nm) where the Kerr gate system was applied to suppress the overlapping fluorescence emission. Peroxide bleached conventional kraft cooking pulps showed a medium intensity band at 1605 cm^{-1} and a low intensity Raman band at 1657 cm^{-1}. The band at 1605 cm^{-1} originates from the aromatic nucleus (Agarwall 1999, pp. 201-205) in the residual chromophoric lignin structures. The band at 1657 cm^{-1}, in turn, originates from C=C and C=O double bond vibrations, and the appearance of this band in the vis-RR spectra is an indication of the presence of chromophoric lignin structures such

as *p*-quinone, coniferaldehyde, or coniferyl alcohol in these pulps. However, coniferyl alcohol does not absorb radiation at 400 nm (Goldschmid 1971) and its contribution to the UVRR spectrum should be relatively small. It is possible though, that coniferyl alcohol contributes to the spectrum in non-enhanced Raman scattering as, for example, celluloses do. Moreover, the contribution to the band at 1657 cm^{-1} from hexenuronic acids is presumed to be minor since hexenuronic acids do not absorb light at 400 nm (Halttunen *et al.* 2001a). The resonance Raman spectra of conventional kraft cooking pulps with chlorine dioxide bleaching contained only the resonance enhanced Raman band at 1605 cm^{-1} originating from the aromatic chromophore structures. There was no indication of other types of chromophoric lignin structures in these pulps.

The square root of the intensity of the aromatic Raman band (1605 cm^{-1}) relative to the intensity of the cellulose band (1098 cm^{-1}) showed good linear correlation with brightness. This is in accordance with the approximated Kubelka-Munk theory on the light scattering and absorption capacity of paper (Leskelä 1998). Linear correlation indicated that the aromatic chromophore band is at least partly due to the residual chromophoric lignin structures. Chlorine dioxide and peroxide bleached pulps showed dissimilar correlations, however, indicating that the chromophores in these pulps are of different nature. Since chlorine dioxide bleached pulp had lower intensity at 1605 cm^{-1} at the same brightness level as peroxide bleached pulp, the chromophores in the chlorine dioxide bleached pulp must have had a greater absorption coefficient at 457 nm (the determination wavelength for brightness) or this pulp must have contained more non-aromatic chromophores, such as *o*-quinones, than the peroxide bleached pulp did. These pulps also exhibited dissimilar correlations between the aromatic to cellulose ratio and the kappa number, additionally indicating the presence of different kinds of chromophores.

7 QUANTIFICATION OF LIGNIN AND HEXA (II, III, V and new data)

The quantification of lignin with Raman spectroscopy has traditionally been based on direct determination of lignin band intensity relative to cellulose band intensity. However, the cellulose band is not resolved in the UVRR spectra of unbleached pulps, as shown in Figure 1. This is because the cellulose bands are too weak to be observed relative to the resonantly enhanced lignin bands. Therefore, the cellulose band cannot serve as a reference band for pulps with lignin contents of about 4-5%.

PLS calibration was used to predict the concentrations of lignin and hexenuronic acid (HexA) groups in differently bleached softwood pulps (II). The PLS method was selected because samples of different concentrations were to be analyzed and, as just noted, the cellulose band was not available for quantifying purposes. The concentration of lignin was predicted relatively accurately with standard error of prediction (SEP) 0.6% (lignin range 0.4–5.6%). The prediction of HexA concentration was not as accurate─SEP was 7.0 mmol/kg (HexA range 4.6-40.9 mmol/kg)─but the accuracy of the determination was increased to SEP of 4.4 mmol/kg (range 14.0-38.1 mmol/kg) by using only pulps from peroxide bleaching sequence with oxygen delignification. The results confirmed that PLS calibration can be used to predict the concentrations of lignin and HexA relatively accurately. It needs to be added, however, that several factors affect the accuracy of the analysis. For example, the calibration was accomplished by using several differently cooked and bleached pulps. Determination of their lignin, HexA and kappa number by the reference methods may contain some errors because the pulps differ in chemical composition, which affect the kappa number and the experimental factors in lignin determination. It was also clear that the prediction ability of the model increased when all samples were of relatively similar concentration. On this basis, it is recommended that, for unknown samples, a wide range model should be used first, and then a narrow range model to predict the concentration more accurately. The method should be applicable to hardwood pulps as well.

The extent of the resonance enhancement of the aromatic lignin structures decreased (III), when visible excitation wavelength was used, enabling the detection of both lignin and cellulose bands in the resonance Raman spectra of unbleached and bleached pulps. This was confirmed by studying pulps with 400 nm excitation wavelength. With this excitation, the residual lignin structures were still resonance or pre-resonance enhanced and low concentrations were detectable. Furthermore, the cellulose band was detected in the vis-RR spectra of unbleached pulp with relatively high lignin concentration. Both lignin and cellulose bands were detected in the lignin range of 0.2-4.3% (corresponding to lignin kappa number range of 1.0-28) as can be seen in Figure 4 in which the vis-RR spectra (400 nm) of unbleached and bleached softwood kraft pulps are shown. The detection of both lignin and cellulose bands would enable the quantification of lignin in both unbleached and bleached pulps directly from the relative band intensities. No earlier study (Ibrahim *et al.* 1997; Sun *et al.* 1997; Agarwal *et al.* 2003) has reported the determination of such a wide range of lignin contents with Raman techniques, mainly due to the lack of an optimal excitation wavelength.

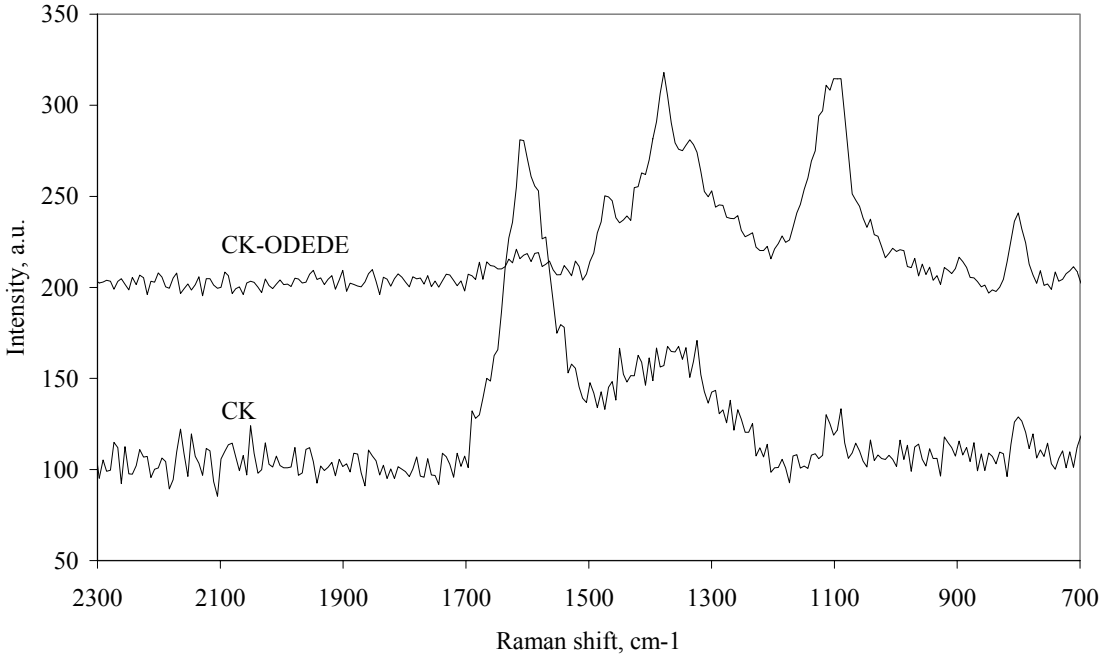

Figure 4. The vis-RR spectra (400 nm) of unbleached and bleached (ODEDE sequence) softwood kraft pulp.

A method for determining lignin directly from UVRR spectra was developed by adding known amounts of kraft lignin to a hardwood pulp with low lignin content (V). Ratios of the intensities of the aromatic lignin and cellulose bands were plotted against the added amount of lignin and a regression line was obtained. The aromatic lignin content of an unknown hardwood pulp could then be calculated from the UVRR band height ratio (aromatic to cellulose) multiplied by a factor of 0.32. This method can be applied to pulps with lignin content less than 5%. The lignin contents of ten bleached hardwood pulps (kappa range 0.8-10.5, lignin range 0.1-0.9%) determined by UVRR correlated with the kappa numbers, even though UVRR is a measure of aromatic lignin and the kappa number measures oxidizable structures. It was also shown that the potassium permanganate used as oxidant in the kappa number determination does not react fully with aromatic and unsaturated structures, and the kappa number for bleached pulps is thus erroneous. Because of the high correlation between the UVRR lignin and kappa number, however, it was

concluded that the kappa number determination is a surprisingly good method even for bleached pulps.

The resonance Raman spectroscopic methods only measure the amount of aromatic lignin in bleached pulps, not other possible residual lignin structures in bleached pulps such as muconic acids and quinones (Alén 2000, pp. 98-99). In bleached pulps, therefore, the actual residual lignin content may be slightly higher than that determined by resonance Raman spectroscopy. Although it is possible to quantify quinones, for example, separately, the C=O and C=C bands appear around 1660 cm^{-1}, where several other compounds, such as hexenuronic acids, may be contributing as well.

8 STUDY OF CARBONYL GROUPS IN CELLULOSIC PULPS (VI)

The nature of the carbonyl groups in cellulosic pulps was studied by comparing results obtained by UVRR spectroscopy and by the CCOA (carbazole-9-carbonyloxyamine) method (Röhrling *et al.* 2002a; Röhrling *et al.* 2002b). An attempt had been made to analyze trace amounts of carbonyls (5-50 μmol/g) by IR spectroscopy (Rosenau 2004), but no differences between samples were detected because the carbonyl contents in the pulps were far below the detection limit in IR. In contrast, clear differences in carbonyl band heights were detected in the UVRR spectra and thus it was feasible to use UVRR spectroscopy to study the carbonyl groups in cellulosic pulps.

CCOA method, just recently introduced for the quantification of trace amounts of carbonyl groups in cellulosic pulps (Röhrling *et al.* 2002a; Röhrling *et al.* 2002b), involves a fluorescence-labeling of the carbonyl groups with a carbazole-9-carbonyloxyamine (CCOA) marker followed by gel permeation chromatographic (GPC) analysis. Information is provided on overall carbonyl content and carbonyl profiles. The concentrations of carbonyl groups determined from the UVRR band height ratios of the unsaturated C=O band at 1655 cm^{-1} and the cellulose band at 1098 cm^{-1} did not correlate with the concentrations determined by the CCOA method. This result was an indication that part of the carbonyls in cellulosic pulps are present in sp^3-hybridized form such as hydrates or hemiacetals rather than in the sp^2-hybridized form of C=O. This was evident because the UVRR measurement detects only carbonyls present in the latter form whereas the CCOA method records the overall carbonyl content. It was also shown that the cellulose carbonyls interchange to carbonyl hydrates or to hemiacetals/hemiketals in the presence of water by studying wetted and dried cellulose pulps by UVRRS. Moreover, the hydrated forms convert back to carbonyls upon drying. This finding underlines the need to perform UVRR measurements of carbonyls at constant wetness.

9 CONCLUSIONS AND SUGGESTIONS

Resonance Raman spectroscopy offers a very useful way of characterizing residual lignin and other unsaturated structures directly from pulp samples. The analysis is relatively fast and nondestructive and it requires only small sample size.

The structural characterization of residual lignin structures is still cumbersome because of the complexity of lignin structures after pulping and bleaching sequences. However, the PLS model based on model compounds (section 6.3) gave interesting and promising results when applied to isolated lignin samples. Further development and use of this model is of great interest. The UVRRS-PLS results correlated significantly with those obtained by existing reference methods and thus, it is reasonable to expect that such models can provide valuable new information about polymeric lignin structures. Further investigation of PLS models and their application in the interpretation of the UVRR spectra not only of lignins but also of unbleached pulps and finally fully bleached pulps would be of great interest. In future, such models might be used to interpret changes in lignin structures during the final stages of bleaching. Other interesting structures that probably could be studied by the UVRRS-PLS method are α–carbonyls, conjugated C=C structures, and methoxyl groups. The results presented may contain some error due to the overlapping of Raman bands in the model compound and lignin spectra and this effect has not been taken into account. The error due to this effect is presumed to be small, however. Attention to the effect of overlapping bands should nonetheless be paid in future.

Chromophoric lignin structures were analyzed by vis-RRS technique, with Kerr gate rejection included to prevent the disturbing fluorescence emission. The fluorescence rejection ratio was estimated at about 250, which means that the Kerr gate effectively rejected the fluorescence emission, enabling the detection of Raman signals. The dissimilar correlations of the vis-RRS results with brightness and kappa number for pulps bleached with chlorine dioxide and with peroxide revealed the presence of dissimilar chromophores in these pulps. Evidently, the different excitation wavelengths give information on different

absorbing groups. There are still many regions in the electromagnetic spectrum that have not been used for the excitation of lignin and pulp samples. The region between 260 and 400 nm has not been investigated, and there are interesting structures absorbing in this region that should be resonantly enhanced in Raman spectroscopy. The interesting lignin structures absorbing in this range include stilbenes, α-carbonyls, coniferyl alcohol, and coniferyl aldehyde.

Lignin and other unsaturated structures can be quantified by UVRR spectroscopy with PLS calibration or from direct band height ratios. If band height ratios are used, the cellulose bands must be distinguishable and this approach cannot, therefore, be utilized for unbleached pulps. Excitation in the visible range (400 nm with Kerr gate rejection), in contrast, allows quantification of lignin in both unbleached and bleached pulps (lignin range 0.2-4.3%, *i.e.* kappa numbers 1.0-28). The direct band height ratio method is more straightforward than the PLS method since the results do not depend on measurement conditions, the pulping and bleaching sequence, or the origin of the sample.

As well, information on the nature of carbonyl groups was obtained by UVRR spectroscopy when the results were compared with those obtained by the CCOA method. Trace amounts of carbonyls (5-50 μmol/g) were easily detected by UVRR spectroscopy, in contrast with IR, which was not sensitive enough. It became clear that the cellulosic carbonyls appear in C=O as well as hydrate and/or hemiacetal forms. Moreover, it was shown that C=O structures interchange to hydrate and/or to hemiacetal/hemiketal form in the presence of water. This study has illustrated the utility of UVRRS in studying trace amounts of unsaturated structures, and shown that detailed structural information can be obtained when the UVRR results are compared with those of another method. In future, more emphasis should be put on comparative studies with other techniques, as in this way more information can be obtained. This is mainly because most techniques provide complementary information with respect to each other and when used together, the advantages of both methods can be utilized.

The main difficulty in the use of resonance Raman techniques is the laborious change in wavelength that is needed to obtain information about different absorbing groups. Moreover, the excitation wavelength cannot usually be selected freely but is restricted to certain values depending on the available laser.

Because Raman analysis can be performed in the presence of water, it can be widely exploited for analytical purposes. Raman probing is possible, for example, in process control in pulp and paper mills. Moreover, this feature diminishes the need for pretreatment before the analysis can be accomplished.

The use of two-dimensional interpretation techniques could be an interesting and useful way to obtain information on lignin structures. This application could be accomplished by comparing the UVRR results obtained with two different wavelengths, 244 nm and 257 nm for example. The interpretation of two-dimensional spectra requires deep understanding of band intensities of different structures and the use of sophisticated interpretation programs. For example, it probably would be possible to distinguish between C=O and C=C structures due to different absorption coefficients at the two different wavelengths.

An interesting approach to obtain fluorescence-free Raman spectra is to collect the spectrum in anti-Stokes mode. The scattering efficiency is significantly lower than in Stokes mode, however, because the vibrational energy levels are significantly less occupied than the lowest energy levels. Scattering efficiency could be facilitated by heating the sample during data collection, but this might cause heat-induced changes to the sample and is thus inconvenient. It may be that the scattering efficiency in anti-Stokes is not high enough to collect decent Raman spectra, at least from solid lignin samples. The occupation of higher vibrational levels is greater in solution state, however, and the collection of anti-Stokes Raman spectra of dissoluted lignin samples might thus be possible. There is a need to develop more advanced anti-Stokes collection devices in order to obtain high collection efficiency of anti-Stokes scattering.

The fluorescence emission, while being a disturbing factor in vis-RRS, may contain useful information about lignin structures. The fluorescence emission is relatively intense compared to Raman scattering. Additionally, it originates mainly from residual lignin structures whereas saturated carbohydrates do not contribute to the fluorescence emission. Therefore, it is reasonable to assume that fluorescence spectroscopy would provide important information about the residual lignin structures. The structural characterization could be accomplished both with conventional fluorescence spectra containing information on different emission wavelengths and with time-resolved fluorescence spectroscopy affording information on fluorescence lifetimes of emitting structures. The first form of fluorescence spectroscopy has been well-investigated in lignin studies, while the latter requires more attention and development of the techniques to detect shorter lifetimes in picosecond scale.

REFERENCES

Agarwal, U.P., An overview of Raman spectroscopy as applied to lignocellulosic materials, in Argyropoulos, D. S., ed., Advances in Lignocellulosics Characterization, TAPPI Press, Atlanta, GA, USA (1999) pp. 201-225.

Agarwal, U.P., Atalla, R.H., FT Raman spectroscopy: What it is and what it can do for research on lignocellulosic materials, 8th International Symposium on Wood and Pulping Chemistry, Helsinki, Finland, Vol. 3 (1995) 67-72.

Agarwal, U.P., Atalla, R.H., In-situ Raman microprobe studies of plant cell walls: Macromolecular organization and compositional variability in the secondary wall of *Picea mariana* (Mill.) B.S.P., Planta, 169 (1986) 325-332.

Agarwal, U.P., Atalla, R.H., Using Raman spectroscopy to identify chromophores in lignin-lignocellulosics, ACS Symposium series 742, Lignin: historical biological, and materials perspectives, Washington, DC, American Chemical Society (2000) Chapt. 11, pp. 250-264.

Agarwal, U.P., Atalla, R.H., Forsskåhl, I., Sequential treatment of mechanical and chemimechanical pulps with light and heat: A Raman spectroscopic study, Holzforschung, 49:4 (1995) 300-312.

Agarwal, U.P., Atalla, R.H., Weinstock, I.A., FT Raman spectroscopy: A rapid, noninvasive technique for direct measurement of lignin in kraft pulp, International Pulp Bleaching Conference, Washington D.C., USA (1996) 531-535.

Agarwal, U.P., Ralph, S.A., FT-Raman spectroscopy of wood: Identifying contributions of lignin and carbohydrate polymers in the spectrum of black spruce (*Picea mariana*), Appl. Spectrosc., 51:11 (1997) 1648-1655.

Agarwal, U.P., Ralph, S.A., Atalla, R.H., FT Raman spectroscopic study of softwood lignin, 9th International Symposium on Wood and Pulping Chemistry, Montreal, Canada (1997) 8-1 – 8-4.

Agarwal, U.P., Weinstock, I.A., Atalla, R.H., FT-Raman spectroscopy for direct measurement of lignin concentrations in kraft pulps, Tappi J., 2:1 (2003) 22-26.

Al-Dajani, W.W., Gellerstedt, G., On the isolation and structure of softwood residual lignin, Nord. Pulp Pap. Res. J., 17:2 (2002) 193-198.

Alén, R., Basic chemistry of wood delignification, in Stenius, P., ed., Forest Products Chemistry, Fapet Oy, Helsinki, Finland (2000).

Angel, S.M., DeArmond, M.K., Hanck, K.W., Wertz, D.W., Computer-controlled instrument for the recovery of a resonance Raman spectrum in the presence of strong luminescence, Anal. Chem., 56 (1984) 3000-3001.

Argyropoulos, D.S., Phosphorus-31 NMR in wood chemistry: A review of recent progress, Res. Chem. Intermed., 21:3-5 (1995) 373-395.

Argyropoulos, D.S., Heitner, C., Morin, F.G., Phosphorus-31 NMR spectroscopy in wood chemistry. Part III. Solid state phosphorus-31 NMR of trimethyl phosphite derivatives of chromophores in mechanical pulp, Holzforschung, 46:3 (1992) 211-218.

Argyropoulos, D.S., Sun, Y., Palus, E., A novel method for isolating residual kraft lignin, 6th European Workshop on Lignocellulosics and Pulp, Bordeaux, France (2000) 23-27.

Atalla, R.H., Raman spectroscopy and the Raman microprobe: Valuable new tools for characterizing wood and wood pulp fibers, J. Wood Chem. Technol., 7:1 (1987) 115-131.

Atalla, R.H., Agarwal, U.P., Raman microprobe evidence for lignin orientation in the cell walls of native woody tissue, Science, 227 (1985) 636-638.

Atalla, R.H., Agarwal, U.P., Recording Raman spectra from plant cell walls, J. Raman Spectrosc., 17 (1986) 229-231.

Atalla, R.H., Agarwal, U.P., Bond, J.S., Raman spectroscopy, in Lin, S.Y., Dence, C.W., eds., Methods in Lignin Chemistry, Springer-Verlag, Berlin, Germany (1992) pp. 162-176.

Atkins, P.W., Physical chemistry, 5th ed., Oxford University press, Great Britain (1994) pp. 591-600.

Azuma, J.-I., Takahashi, N., Koshijima, T., Isolation and characterisation of lignin-carbohydrate complexes from milled-wood lignin fraction of *Pinus densiflora* sieb. et zucc., Carbohydr. Res., 93:1 (1981) 91-104.

Backa, S., Brolin, A., Determination of pulp characteristics by diffuse reflectance FTIR, Tappi J., 74:5 (1991) 218-226.

Bell, S.E.J., Bourguignon, E.S.O., Dennis, A., Analysis of luminescent samples using subtracted shifted Raman spectroscopy, Analyst, 123 (1998) 1729-1734.

Berben, S.A., Rademacher, J.P., Sell, L.O., Easty, D.B., Estimation of lignin in wood pulp by diffuse reflectance Fourier-transform infrared spectrometry, Tappi J., 70:11 (1987) 129-133.

Björkman, A., Studies on finely divided wood, Part I. Extraction of lignin with neutral solvents, Svensk Papperstidn., 59 (1956) 477-485.

Bolker, H.I., Somerville, N.G., Ultraviolet spectroscopic studies of lignins in the solid state. I. Isolated lignin preparations, Tappi, 45 (1962) 826-829.

Bowman, W.D., Spiro, T.G., Fluorescence-free resonance Raman spectra of reduced nicotinamide adenine dinucleotide via ultraviolet excitation, J. Raman Spectrosc., 9 (1980) 369-371.

Brunow, G., Lundquist, K., Gellerstedt, G., Lignin in Sjöström, E., Alén, R., eds., Analytical Methods in Wood Chemistry, Pulping and Papermaking, Springer-Verlag, Berlin, Germany (1999) pp. 77-124.

Chang, H.-m., Isolation of lignin from pulp, in Lin, S.Y., Dence, C.W., eds., Methods in Lignin Chemistry, Springer-Verlag, Berlin, Germany (1992) pp. 71-74.

Deffontaine, A., Delhaye, M., Bridoux, M., Pulsed multichannel Raman technique, in Laubereau, A., Stockburger, M., eds., Time-Resolved Vibrational Spectroscopy, Springer-Verlag, Berlin, Germany (1985) pp. 20-24.

Dence, C.W., The determination of lignin, in Lin, S.Y., Dence, C.W., eds., Methods in Lignin Chemistry, Springer-Verlag, Berlin, Germany (1992) pp. 33-61.

Ehrhardt, S.M., An investigation of the vibrational spectra of lignin model compounds, Ph.D. thesis, The Institute of Paper Chemistry, Appleton, Wisconsin, USA (1984).

Evans, P.A., Differentiating “hard” and “soft” woods using Fourier transform infrared and Fourier transform Raman spectroscopy, Spectrochim. Acta A, 47: 9/10 (1991) 1441-1447.

Everall, N., Hahn, T., Matousek, P., Parker, A.W., Towrie, M., Picosecond time-resolved Raman spectroscopy of solids: Capabilities and limitations for fluorescence rejection and the influence of diffuse reflectance, Appl. Spectrosc., 55 (2001) 1701-1708.

Faix, O., Fourier transform infrared spectroscopy, in Lin, S.Y., Dence, C.W., eds., Methods in Lignin Chemistry, Springer-Verlag, Berlin, Germany (1992) pp. 83-109.

Faix, O., Andersons, B., Zakis, G., Determination of carbonyl groups of six Round Robin lignins by modified oximation and FTIR spectroscopy, Holzforschung, 52:3 (1998) 268-274.

Faix, O., Argyropoulos, D.S., Robert, D., Neirinck, V., Determination of hydroxyl groups in lignins: Evaluation of ^{1}H-, ^{13}C-, ^{31}P-NMR, FTIR and wet chemical methods, Holzforschung, 48:5 (1994) 387-394.

Faix, O., Böttcher, J.H., Determination of phenolic hydroxyl group contents in milled wood lignins by FTIR spectroscopy applying partial least-squares (PLS) and principal components regression (PCR), Holzforschung, 47:1 (1993) 45-49.

Faix, O., Grünwald, C., Beinhoff, O., Determination of phenolic hydroxyl group content of milled wood lignins (MWL's) from different botanical origins using selective aminolysis, FTIR, 1H-NMR, and UV spectroscopy, Holzforschung, 46:5 (1992) 425-432.

Fujii, T., Kamogawa, K., Kitagawa, T., Observation of resonance Raman spectra of S_1, T_1, and S_0 pyrene in solution: Application of a fluorescence rejection technique, Chem. Phys. Lett., 148 (1988) 17-20.

Friese, M.A., Banerjee, S., Lignin determination by FT-IR, Appl. Spectrosc., 46:2 (1992) 246-248.

Gellerstedt, G., Pranda, J., Lindfors, E.-L., Structural and molecular properties of residual birch kraft lignins, J. Wood Chem. Technol., 14:4 (1994) 467-482.

Gil, A.M., Neto, C.P., Solid-state NMR studies of wood and other lignocellulosic materials, Ann. Rep. NMR spectrosc., 37 (1999) 75-117.

Gilarranz, M.A., Rodriguez, F., Oliet, M., Garcia, J., Alonso, V., Phenolic OH group estimation by FTIR and UV spectroscopy. Application to organosolv lignins, J. Wood Chem. Technol., 21:4 (2001) 387-395.

Goldschmid, O., Ultraviolet spectra in Sarkanen, K.V., Ludwig, C.H., eds., Lignins - Occurrence, Formation, Structure and Reactions, Wiley, New York, USA (1971) pp. 250-252.

Grandmaison, J.L., Thibault, J., Kaliaguine, S., Chantal, P.D., Fourier transform infrared spectrometry and thermogravimetry of partially converted lignocellulosic materials, Anal. Chem., 59:17 (1987) 2153-2157.

Hage, E.R.E. van der, Mulder, M.M., Boon, J.J., Structural characterization of lignin polymers by temperature-resolved in-source pyrolysis-mass spectrometry and Curie-poin pyrolysis-gas chromatography/mass spectrometry, J. Anal. Appl. Pyrolysis, 25 (1993) 149-183.

Halttunen, M., Jääskeläinen, A.-S., Löija, M., Perander, A.-M., Vyörykkä, J., Vuorinen, T., Evaluation of pulping and bleaching processes with novel spectroscopic techniques, 7th Brazilian Symposium on the Chemistry of Lignins and Other Wood Components, Belo Horizonte, Brazil (2001b) 91-98.

Halttunen, M., Vyörykkä, J., Hortling, B., Tamminen, T., Batchelder, D., Zimmermann, A., Vuorinen, T., Study of residual lignin in pulp by UV resonance Raman spectroscopy, Holzforschung, 55:6 (2001a) 631-638.

Harris, J.M., Chrisman, R.W., Lytle, F.E., Tobias, R.S., Sub-nanosecond time-resolved rejection of fluorescence from Raman spectra, Anal. Chem., 48 (1976) 1937-1943.

Hase, T., Tables for organic spectrometry, Otatieto Oy, Helsinki, Finland (1992) pp. 15.

Hatcher, P.G., Chemical structural studies of natural lignin by dipolar dephasing solid-state carbon-13 nuclear magnetic resonance, Org. Geochem., 11:1 (1987) 31-39.

Hatfield, G.R., Maciel, G.E., Erbatur, O., Erbatur, G., Qualitative and quantitative analysis of solid lignin samples by carbon-13 nuclear magnetic resonance spectrometry, Anal. Chem., 59:1 (1987) 172-179.

Hawkes, G.E., Smith, C.Z., Utley, J.H.P., Vargas, R.R., Viertler, H., A comparison of solution and solid state ^{13}C NMR spectra of lignins and lignin model compounds, Holzforschung, 47:4 (1993) 302-312.

Hortling, B., Tamminen, T., Kenttä, E., Determination of carboxyl and non-conjugated carbonyl groups in dissolved and residual lignins by IR spectroscopy, Holzforschung, 51:5 (1997) 405-410.

Howard, J., Everall, N.J., Jackson, R.W., Hutchinson, K., Fluorescence rejection in Raman spectroscopy using a synchronously pumped, cavity-dumped dye laser and gated photon counting, J. Phys. E: Sci. Instrum., 19:11 (1986) 934-943.

Ibrahim, A., Oldham, P.B., Conners, T.E., Schultz, T.P., Rapid characterization of wood pulp lignin by Fourier transform Raman spectroscopy, Microchem. J., 56:3 (1997) 393-402.

Isogai, A., Ishizu, A., Nakano, J., Residual lignin in unbleached kraft pulp. Part 2. Analysis of unbleached kraft pulp by new permethylation method, J. Wood Chem. Technol., 7:4 (1987) 463-483.

Jääskeläinen, A.-S., Saariaho, A.-M., Matousek, P., Towrie, M., Parker, A. W., Vuorinen, T., Characterisation of residual lignin structures by UV resonance Raman spectroscopy and the possibilities of Raman spectroscopy in the visible region with Kerr-gated fluorescence suppression, 12th International Symposium on Wood and Pulping Chemistry, Madison, WI, USA, Vol. 1 (2003) 139-142.

Kenton, R.C., Rubinovitz, R.L., FT-Raman investigations of forest products, Appl. Spectrosc., 44:8 (1990) 1377-1380.

Kihara, M., Takayama, M., Wariishi, H., Tanaka, H., Determination of the carbonyl groups in native lignin utilizing Fourier transform Raman spectroscopy, Spectrochim. Acta A, 58 (2002) 2213-2221.

Leary, G.J., Newman, R.H., Cross polarization/magic angle spinning nuclear magnetic resonance (CP/MAS NMR) spectroscopy, in Lin, S.Y., Dence, C.W., eds., Methods in Lignin Chemistry, Springer-Verlag, Berlin, Germany (1992) pp. 146-161.

Leskelä, M., Optical properties, in Niskanen, K., ed., Paper physics, Fapet Oy, Helsinki, Finland (1998) pp. 117-137.

Li, C., Stair, P.C., Ultraviolet Raman spectroscopy characterization of coke formation in zeolites, Catal. Today, 33 (1997) 353-360.

Li, J., Gellerstedt, G., The contribution to kappa number from hexenuronic acid groups in pulp xylan, Carbohydr. Res., 302:3-4 (1997) 213-218.

Li, J., Sevastyanova, O., Gellerstedt, G., The relationship between kappa number and oxidizable structures in bleached pulps, J. Pulp Pap. Sci., 28:8 (2002) 262-266.

Liitiä, T., Application of modern NMR spectroscopic techniques to structural studies of wood and pulp components, Ph.D. Thesis, University of Helsinki, Finland (2002).

Liitiä, T., Maunu, S.L., Hortling, B., Solid state NMR studies of residual lignin and its association with carbohydrates, J. Pulp Pap. Sci., 26:9 (2000) 323-330.

Liitiä, T., Maunu, S.L., Hortling, B., Solid state NMR studies on inhomogeneous structure of fibre wall in kraft pulp, Holzforschung, 55:5 (2001) 503-510.

Liitiä, T., Maunu, S.L., Sipilä, J., Hortling, B., Application of solid-state ^{13}C NMR spectroscopy and dipolar dephasing technique to determine the extent of condensation in technical lignins, Solid State NMR, 21:3/4 (2002) 171-186.

Lundquist, K., Proton (^{1}H) NMR spectroscopy, in Lin, S.Y., Dence, C.W., eds., Methods in Lignin Chemistry, Springer-Verlag, Berlin, Germany (1992) pp. 242-249.

Manders, W.F., Solid-state ^{13}C NMR determination of the syringyl/guaiacyl ratio in hardwoods, Holzforschung, 41:1 (1987) 13-18.

Martínez, A.T., Almendros, G., Gonzalez-Vila, F.J., Frund, R., Solid-state spectroscopic analysis of lignins from several austral hardwoods, Solid State NMR, 15:1 (1999) 41-48.

Mateo, C., Chirat, C., Lachenal, D., Saariaho, A.-M., Vuorinen, T., What are the Chromophores left at the end of a bleaching process?, 7th European Workshop on Lignocellulosics and Pulp, Turku, Finland (2002) 19-22.

Matousek, P., Towrie, M., Stanley, A., Parker, A.W., Efficient rejection of fluorescence from Raman spectra using picosecond Kerr gating, Appl. Spectrosc., 53:12 (1999) 1485-1489.

Matousek, P., Towrie, M., Ma, C., Kwok, W.M., Phillips, D., Toner, W.T., Parker, A.W., Fluorescence suppression in resonance Raman spectroscopy using a high-performance picosecond Kerr gate, J. Raman Spectrosc., 32:12 (2001) 983-988.

Maunu, S.L., NMR studies of wood and wood products, Prog. NMR Spectrosc., 42:2 (2002) 151-174.

Meier, D., Faix, O., Pyrolysis-gas chromatography-mass spectrometry, in Lin, S.Y., Dence, C.W., eds., Methods in Lignin Chemistry, Springer-Verlag, Berlin, Germany (1992) pp. 177-199.

Milne, T.A., Chum, H.L., Agblevor, F., Johnson, D.K., Standardized analytical methods, Biomass and Bioenergy, 2:1-6 (1992) 341-366.

Mosier-Boss, P.A., Lieberman, S.H., Newbery, R., Fluorescence rejection in Raman spectroscopy by shifted spectra, edge detection, and FFT filtering techniques, Appl. Spectrosc., 49:5 (1995) 630-638.

Nuopponen, M., Saariaho, A-M., Vuorinen, T., A study on resin acid model compounds and resin in pine wood with UV resonance Raman (UVRR) spectroscopy, 7th European Workshop on Lignocellulosics and Pulp, Turku, Finland (2002) 221-224.

Nuopponen, M., Vuorinen, T., Jämsä, S., Viitaniemi, P., Thermal modifications in softwood studied by FT-IR and UV resonance Raman spectroscopies, J. Wood Chem. Technol., 24:1 (2004) 13-26.

Olmstead, J.A., Gray, D.G., Fluorescence spectroscopy of cellulose, lignin and mechanical pulps: A review, J. Pulp Pap. Sci., 23:12 (1997) 571-581.

Ona, T., Sonoda, T., Ito, K., Shibata, M., Katayama, T., Kato, T., Ootake, Y., Non-destructive determination of lignin syringyl/guaiacyl monomeric composition in native wood by Fourier transform Raman spectroscopy, J. Wood Chem. Technol., 18:1 (1998) 43-51.

Ona, T., Sonoda, T., Ito, K., Shibata, M., Kato, T., Ootake, Y., Non-destructive determination of wood constituents by Fourier transform Raman spectroscopy, J. Wood Chem. Technol., 17:4 (1997) 399-417.

Ona, T., Sonoda, T., Ito, K., Shibata, M., Kato, T., Ootake, Y., Tamai, T., Kojima, Y., Rapid prediction of native wood pulp properties by Fourier transform Raman spectroscopy, J. Pulp Pap. Sci., 26:2 (2000) 43-47.

Perander, A-M., Halttunen, M., Jääskeläinen, A.-S., Vyörykkä, J., Vuorinen, T., Determination of Residual Lignin from Pulp Samples with UV Resonance Raman Spectroscopy, 11th International Symposium on Wood and Pulping Chemistry, Nice, France, Vol. 1 (2001) 331-334.

Pew, J.C., Properties of powdered wood and isolation of lignin by cellolytic enzymes, Tappi, 40 (1957) 553-558.

Ramos, L.P., Mathias, A.L., Silva, F.T., Cotrim, A.R., Ferraz, A.L., Chen, C.-L., Characterization of residual lignin after SO_2-catalyzed steam explosion and enzymatic hydrolysis of eucalyptus viminalis wood chips, J. Agric. Food Chem., 47:6 (1999) 2295-2302.

Reis Machado, A.S., Sardinha, R.M.A., Gomes De Azevedo, E., Nunes Da Ponte, M., Characterization of residues and extracts of high-pressure extraction of eucalyptus wood by 1,4-dioxane-CO_2 mixtures. Part 1. Characterization by FTIR, UV, and HPLC, Holzforschung, 50:6 (1996) 531-540.

Río, J.C. del, Gutiérrez, A., Romero, J., Martínez, M.J., Matrínez, A.T., Identification of residual lignin markers in eucalypt kraft pulps by Py-GC/MS, J. Anal. Appl. Pyrolysis, 58-59 (2001) 425-439.

Robert, D., Carbon-13 nuclear magnetic resonance spectrometry, in Lin, S.Y., Dence, C.W., eds., Methods in Lignin Chemistry, Springer-Verlag, Berlin, Germany (1992) pp. 250-273.

Rodrigues, J., Meier, D., Faix, O., Pereira, H., Determination of tree to tree variation in syringyl/guaiacyl ratio of *Eucalyptus globulus* wood lignin by analytical pyrolysis, J. Anal. Appl. Pyrolysis, 48:2 (1999) 121-128.

Rosenau, T., unpublished data, written communication, 17th June (2004).

Röhrling, J., Potthast, A., Rosenau, T., Lange, T., Borgards, A., Sixta, H., Kosma, P., A novel method for the determination of carbonyl groups in cellulosics by fluorescence labeling. Part II: Validation and applications, Biomacromolecules, 3:5 (2002a) 969-975.

Röhrling, J., Potthast, A., Rosenau, T., Lange, T., Ebner, G., Sixta, H., Kosma, P., A novel method for the determination of carbonyl groups in cellulosics by fluorescence labeling. Part I: Method development. Biomacromolecules, 3:5 (2002b) 959-968.

Sands, H.S., Hayward, I.P., Kirkbride, T.E, Bennett, R., Lacey, R.J., Batchelder, D.N., UV-excited resonance Raman spectroscopy of narcotics and explosives, J. Forensic Sci., 43:3 (1998) 509-513.

SCAN-C 1:00, Kappa number; Scandinavian Pulp, Paper and Board Testing Committee.

Schultz, T.P., Burns, D.A., Rapid secondary analysis of lignocellulose: Comparison of near infrared (NIR) and Fourier transform infrared (FTIR), Tappi J., 73:5 (1990) 209-212.

Schultz, T.P., Templeton, M.C., McGinnis, G.D., Rapid determination of lignocellulose by diffuse reflectance Fourier transform infrared spectrometry, Anal. Chem., 57:14 (1985) 2867-2869.

Seca, A.M.L., Cavaleiro, J.A.S., Domingues, F.M.J., Silvestre, A.J.D., Evtuguin, D. Pascoal, N.C., Structural characterization of the lignin from the nodes and internodes of Arundo donax reed, J. Agric. Food Chem., 48:3 (2000) 817-824.

Shreve, A.P., Cherepy, N.J., Mathies, R.A., Effective rejection of fluorescence interference in Raman spectroscopy using a shifted excitation difference technique, Appl. Spectrosc., 46:4 (1992) 707-711.

Sjöholm, E., Gustafsson, K., Colmsjö, A., Size-exclusion chromatography of lignins using lithium chloride/N,N-dimethylacetamide as mobile phase. I. Dissolved and residual birch kraft lignins, J. Liq. Chrom. Rel. Technol., 22:11 (1999) 1663-1685.

Sjöholm, R., Basics of NMR spectroscopy, Ph.D. course material on Advanced analytical methods related to pulping and papermaking, Åbo Akademi, Turku, Finland, 14-18 May (2001).

Sonoda, T., Ona, T., Yokoi, H., Ishida, Y., Ohtani, H., Tsuge, S., Quantitative analysis of detailed lignin monomer composition by pyrolysis-gas chromatography combined with preliminary acetylation of the samples, Anal. Chem., 73:22 (2001) 5429-5435.

Sukhov, D.A., Evstigneyev, E.I., Derkacheva, O.Y., Nabiev, I.R., Kuptsov, A.H., Raman spectroscopy of lignin and model compounds, 7th International Symposium of Wood and Pulping Chemistry, Beijing, China, Vol. 2 (1993) 969-974.

Sukhov, D.A., Evstigneyev, E.I., Feofanov, A.V., Valov, P.M., Terentyev, O.A., UV-Raman resonance spectroscopy – new method to analyse residual lignin, 1st European Workshop on Lignocellulosics and Pulp, Hamburg, Germany (1990) 367-373.

Sun, Z., Ibrahim, A., Oldham, P.B., Schultz, T.P., Conners, T.E., Rapid lignin measurement in hardwood pulp samples by near-infrared Fourier transform Raman spectroscopy, J. Agric. Food Chem., 45:8 (1997) 3088-3091.

Tahara, T., Hamaguchi, H., Picosecond Raman spectroscopy using a streak camera, Appl. Spectrosc., 47 (1993) 391-398.

Takayama, M., Johjima, T., Yamanaka, T., Wariishi, H., Tanaka, H., Fourier transform Raman assignment of guaiacyl and syringyl marker bands for lignin determination, Spectrochim. Acta A, 53A:10 (1997) 1621-1628.

Takei, T., Kato, N., Iijima, T., Higaki, M., Raman spectroscopic analysis of wood and bamboo lignin, Mokuzai Gakkaishi, 41:2 (1995) 229-236.

TEKES, The National Technology Agency of Finland, Hiilihydraatit prosessiteollisuudessa –loppuraportti, in Finnish, (1996) pp. 48.

Teleman, A., Harjunpää, V., Tenkanen, M., Buchert, J., Hausalo, T., Drakenberg, T., Vuorinen, T., Characterization of 4-deoxy-β-L-threo-hex-4-eno-pyranosyluronic acid attached to xylan in pine kraft pulp and pulping liquor by 1H NMR and 13 C NMR spectroscopy, Carbohydr. Res., 272:1 (1995) 55-71.

Wegener, G., Strobel, C., Determination of phenolic hydroxyl groups in lignins and lignin fractions by means of FTIR spectroscopy, 2nd Brazilian Symposium on the Chemistry of Lignins and Other Wood Components, Brazil (1991) 32-43.

Weinstock, I.A., Atalla, R.H., Agarwal, U.P., Minor, J.L., Petty, C., Fourier transform Raman spectroscopic studies of a novel wood pulp bleaching system, Spectrochim. Acta A, 49:5/6 (1993a) 819-829.

Weinstock, I.A., Minor, J.L., Reiner, R.S., Agarwal, U.P., Atalla, R.H., FT Raman and UV visible spectroscopic studies of a highly selective polyoxometalate bleaching system, Tappi proc., Pulping Conference (1993b) 519-532.

Willard, H.H., Merritt Jr., L.L., Dean, J.A., Settle Jr., F.A., Instrumental Methods of Analysis, Wadsworth, Inc. Belmont, CA, USA (1988).

Yamasaki, T., Hosoya, S., Chen, C.-L., Gratzl, J.S., Chang, H.-m., Characterization of residual lignin in kraft pulp, 1st International Symposium on Wood and Pulping Chemistry, Stockholm, Sweden, Vol. 2 (1981) 34-42.

Yaney, P.P., Reduction of fluorescence background in Raman spectra by the pulsed Raman technique, J. Opt. Soc. Am. 62:11 (1972) 1297-1303.