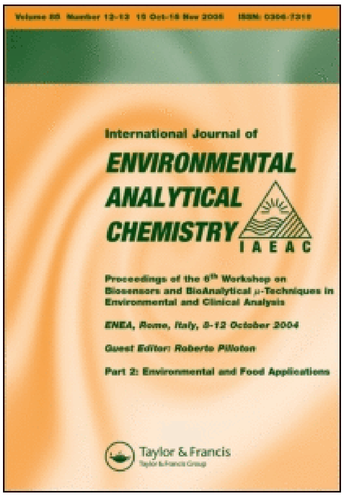

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Characterization of Chlorolignins Compared with Humic Substances Using ^{13}C and ^{1}H NMR and IR Spectroscopy

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CHARACTERIZATION OF CHLOROLIGNINS COMPARED WITH HUMIC SUBSTANCES USING ^{13}C AND ^{1}H NMR AND IR SPECTROSCOPY

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Infrared, solid-state ^{13}C NMR and liquid-state ^{1}H NMR spectra were recorded as a means of investigating structural components in the high-molecular part of pulp mill effluent and different stages of the chlorine bleaching process. Comparison was made with the spectra of kraft lignin, native lignin, and humic substances. The ^{1}H and ^{13}C spectra of mill sample fractions showed that there are no marked differences in the structure of high-molecular-weight fractions before and after the purification plant. However, comparison of the ^{13}C spectra of mill samples with the spectra of native and kraft lignin showed that lignin undergoes a pronounced change in the bleaching process. The ^{13}C NMR spectrum of the purification plant outflow sample closely resembled the spectra of the humic reference materials. The most significant difference in the infrared spectra of the chlorolignin fractions and reference humic materials was the presence of strong C-Cl absorption bands in the chlorolignin spectra and their absence from the spectra of humic samples.

KEY WORDS: Chlorolignins, humic substances, NMR spectroscopy, infrared spectroscopy, spent bleach liquors, structural characterization

INTRODUCTION

Chlorine bleaching of cellulose pulp is a significant source of pollution of natural waters. During conventional bleaching of chemical pulp, chlorinated compounds are formed primarily out of the residual lignin but also out of the carbohydrates and extractives.[1] Most of the organically bound chlorine is found in the high-molecular-weight fraction (MW>1000) of the effluent known as chlorolignin.[1] The structures of these biopolymers, as well as changes in structures occurring during bleaching processes and treatment in biological purification plants are poorly understood. Studies of structures and the fate of chlorolignins in the aquatic environment are needed to increase our understanding of the environmental chemistry of pulp mill effluents.

The major part of the dissolved organic matter in natural waters consists of acidic polymers generally classified as humic substances. These substances have physical, chemical and biological effects on the environment. For example, they complex trace metals and organic compounds, have buffer capacity, and influence assimilation by absorbing light.[2] The combined effects of humic substances and chlorolignins in water bodies are not known.

NMR and IR spectroscopy have been widely used in the characterization of the structural units in different types of lignin preparations and humic substances. Solid-state ^{13}C NMR and IR methods are non-destructive and provide direct information about the chemical nature of structural components. Modern NMR multipulse techniques have enabled investigators to gather considerable information about lignins and humic matter. Solid-state ^{13}C NMR spectroscopy has been used to analyze the structures of native wood lignin,[3,4] various lignin preparations, [3-6] and kraft lignin model (Indulin AT-R),[6,7] while liquid-state ^{13}C NMR spectroscopy, modern pulse programmes, and model compounds have been applied to the study of native lignin[8-12] and the characterization of kraft lignins[13-15] and spent bleach liquors.[16-18] Lignin preparations and spent liquors of pulp mills have also been analyzed by modern ^{1}H NMR spectroscopy.[18-20] Structural analyses of humic substances have been made by both liquid and solid-state NMR from soils, water, and sediment.[21-28] And in at least one study a comparison has been made of native lignins, kraft lignins, and humic substances of different origin.[29]

Infrared spectra of humic substances of terrestrial and aquatic origin have been measured as a means of characterizing humic structure.[30-34] IR spectroscopy has also been applied to the analysis of native lignin[3,35,36], kraft lignin,[36] and spent bleach liquors.[16,17]

Our earlier ^{1}H NMR spectroscopic studies[37,38] and other work[39,40] have shown that most of the water-soluble chlorolignin in receiving waters of kraft pulp mill is structurally very similar to natural humic substances.

The aim of the present study was to investigate further the structure of the high-molecular part of pulp mill effluent and the structure of the effluent from different stages of the chlorobleaching process. Infrared, solid-state ^{13}C NMR, and liquid-state ^{1}H NMR spectroscopy were used for analyses of the structural components, and comparisons were made with kraft lignin, native lignin, and humic substances.

EXPERIMENTAL

Sample preparation

Mill samples Samples were obtained from a Finnish kraft softwood pulp mill using a five-stage (D+C)EDED bleaching sequence (D+C = chlorine dioxide + chlorine treatment, E = alkaline extraction stage and D = chlorine dioxide treatment) and activated-sludge treatment for the total effluent. Effluent waters were taken at four sampling points: the (D+C) stage (sample DC), the E stage (sample E), and the inflow (sample IN) and the outflow (sample OUT) streams of the biological purification plant (activated sludge). Effluent waters were centrifuged and filtered through a membrane filter (0.45 μ, Millipore, Bedford, MA, USA). The pH of the water samples was adjusted to 10.0 to prevent the formation of hydrogen bridges which cause aggregation.

Reference samples The following lignin and humic preparations were used: birch lignin (LIGN) isolated by extraction with alkali followed with acidification; commercial kraft pine lignin (Indulin AT-R) from the Westvaco Co. (sample IND); Nordic humic acid (NoHA) and fulvic acid (NoFA) references prepared from the runoff water from the Norwegian humic lake Hellerrudmyra by the XAD-8 resin method.[42]

Ultrafiltration One-liter samples (DC, E, IN and OUT) were ultrafiltrated using a Millipore Pellicon Laboratory Cassette System (Millipore). The first separation was carried out using a Millipore PTTK membrane with nominal-molecular-weight (NMW) cutoff value of 30,000 Da. After the sample water had been concentrated to 50 ml, 3 × 150 ml of deionized water were used to wash (dilute with water and concentrate again) the concentrate. The retentate (NMW>30,000 Da, fraction 1) was saved for further treatment and filtrate (NMW<30,000 Da) was submitted to a second ultrafiltration using a PTGC membrane with a NMW cutoff of 10,000 Da. The ultrafiltration was carried out as described above and repeated once more with a PCAC membrane (NMW cutoff 1000 Da). The three high-molecular-weight fractions (1 NMW > 30,000 Da; 2 NMW 10,000-30,000 Da and 3 NMW 1,000-10,000 Da) thus obtained for each sample (DC, E, IN and OUT) were freeze-dried (DR Morand, Miniloy II, 3005) and the residual masses weighed. The samples were stored frozen for further analysis.

Analysis of the samples

Infrared spectra IR spectra of chlorolignin fractions and reference materials were recorded on a Perkin-Elmer Model 683 spectrometer using the KBr-pellet technique. The sample concentration was 0.6-1.5%.

NMR measurements The liquid-state ^{1}H NMR spectra were acquired using a JEOL GSX-270 spectrometer operating at 270 MHz and equipped with a 5 mm dual (C/H) probe. Instrumental conditions used were ambient temperature, 90° flip angle, pulse repetition time 2 s, spectral width 5500 Hz, data points for FID 65 K, digital resolution 0.08 Hz and 10,000 pulses per measurement. Samples were dissolved in D_2O; sample concentrations were 2.0-3.7% (w/w) for chlorolignin fractions and 0.3-0.7% (w/w) for reference materials. The chemical shift of the solvent signal (D_2O 4.8 ppm) was used to set the scale to TMS=0. The proton spectra showed three distinct groups of signals (I, aliphatic; II, carbohydrate; III, olefinic/aromatic protons). These three areas were integrated gravimetrically.

The solid-state ^{13}C NMR spectra were acquired using freeze-dried samples and cross-polarization magic angle spinning (CP/MAS) at 3.3 kHz. The CP/MAS spectra were recorded on a JEOL FX-200 spectrometer operating at 50.4 MHz. Instrumental conditions used were contact time 1 ms, repetition time 1 s, spectral width 15 kHz, line broadening 40 Hz, and number of scans from 6,000 to 15,000. The CP/MAS spectra showed four distinct groups of carbon signals (I, aliphatic; II, carbohydrate; III, olefinic/aromatic; IV, carboxylic carbons). These four areas were integrated gravimetrically.

TOCl determination Total amount of organically bound chlorine (TOCl) was determined using the wet combustion method.[41]

Elemental analyses Elemental analyses (C, N and H) were performed using various combustion techniques with a Carlo Erba Elemental Analyzer at the University of Joensuu. The chlorine content had been determined using TOCl analysis (see above). The residual from the 100% was assumed to be the summed content of oxygen and sulfur.

RESULTS AND DISCUSSION

Sample data and elemental compositions of samples are presented in Table 1. Relative amounts (cumulative percentages) of organic matter (OM) in the three molecular size fractions of the ultrafiltrates (NMW < 1000 Da) from each sample are given in Figure 1.

The model lignin and humic samples were less oxygenated (had higher carbon and lower O+S contents) than the chlorolignins (Table 1). Organic chlorine (OCl) contents in model lignin and humic samples were below the limit of determination (< 0.1%). OCl and its ratio to organic matter (OCl/OM) in chlorolignins were slightly higher in the first chlorination (D/C stage) waste than in other stages and independent of molecular size.

As can be seen in Figure 1, the average size of macromolecules was significantly smaller in the D/C than the E stage. This is a consequence of the low pH in the D/C and high pH in

Table 1 Elemental analysis and masses of NMW fractions and reference materials.

Sample code	*NMW (KDa)*	*OM (mg/l)*	*C (%)*	*H (%)*	*N (%)*	*O+S (%)*	*OCl (%)*	*OCl/OM (%)*
DC1	>30	50.6	30.4	3.9	0.2	60.6	4.9	
DC2	10-30	93.5	35.7	3.9	0.2	53.1	7.1	6.1
DC3	1-10	232.5	33.3	3.7	0.2	56.4	6.4	
E1	>30	883.4	41.6	4.1	0.2	48.9	5.2	
E2	10-30	917.9	37.1	3.6	0.2	54.8	4.3	4.4
E3	1-10	446.3	36.3	3.6	0.2	56.1	4.3	
IN1	>30	239.7	38.8	4.1	0.6	52.0	4.5	
IN2	10-30	264.2	37.5	3.9	0.3	53.5	4.8	4.4
IN3	1-10	247.8	36.5	3.7	0.3	55.5	4.0	
OUT1	>30	168.4	39.6	4.3	0.5	51.7	3.9	
OUT2	10-30	195.7	36.9	4.0	0.3	54.7	4.1	3.7
OUT3	1-10	155.9	34.7	3.7	0.3	58.1	3.2	
IND	-	-	63.3	5.7	0.3	30.7	-	
LIGN.	-	-	54.0	5.6	0.3	40.1	-	-
NOFA*	XAD	-	49.4	3.4	0.9	46.3	-	
NOHA*	XAD	-	50.6	3.7	1.4	44.4	-	

*Reference samples NoFa and NoHA were isolated from water (Norway 1986) by XAD-8 method.[42] Elemental analysis from ref. 43.

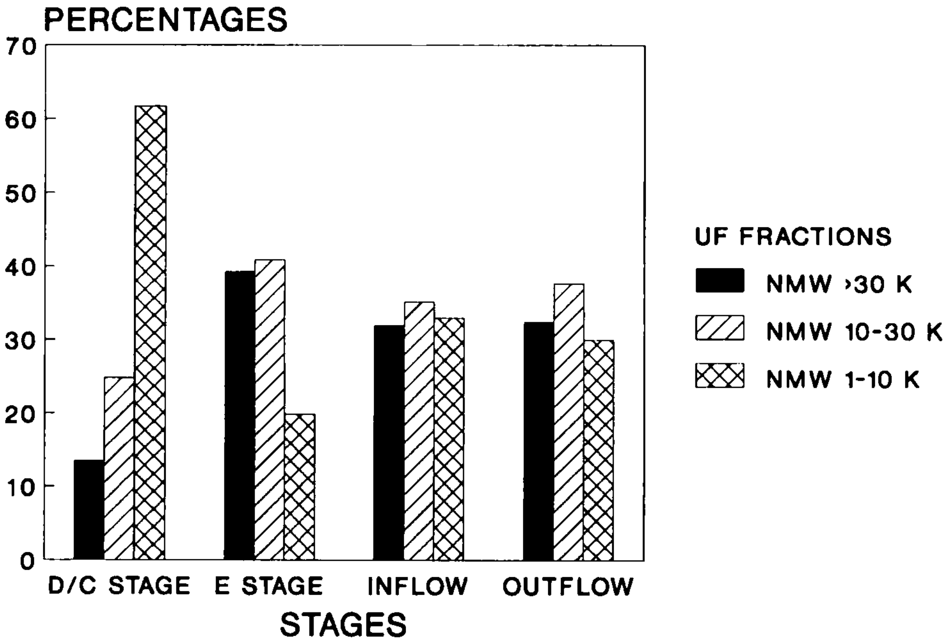

Figure 1 Relative amounts of the three high-molecular fractions in samples DC (D/C stage), E (E stage), IN (inflow), and OUT (outflow) (OM values in Table 1) shown as percentage mass distribution.

the E stage. The formation of phenolate and carboxylate anion groups in chlorolignin under alkaline extraction conditions makes the higher molecular weight fraction more water-soluble in the E stage.

The distribution of the organically bound chlorine (OCI) in NMW fractions is in good agreement with another study.[44] The ratio of OCI to OM in the high molecular parts of the chlorolignin samples decreased slightly from D/C to E and from inflow to outflow (Table 1). Further decrease of OCI/OM in the recipient sediments downstream the discharge point is illustrated in Figure 2.[45]

The moderately steep gradient in the plot of OCI/OM vs. distance from the discharge point (Figure 2) confirms that the main part of the OCI in sediments originates from the pulp mill. Dilution downstream is explained partly by degradation of the OCI and partly by dilution with other sources of OM.

Both chlorolignin and humic substances are heterogeneous macromolecular materials. This means that the specific resonance regions of the different structural features of the compounds are overlapping in infrared, liquid-state ^{1}H, and solid-state ^{13}C NMR spectra complicating assignments and reducing the usefulness of the spectra for structure-analytical determinations.

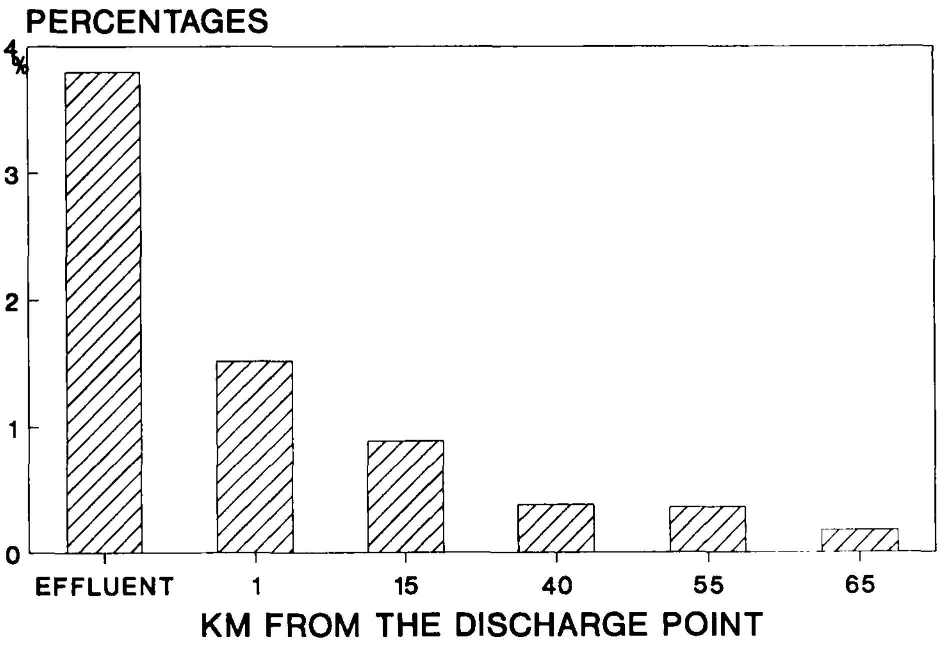

Figure 2 Ratio of OCl to OM in effluent (this work) and in bottom sediments of the recipient lakes at different distances from the discharge point expressed in per cent.[45].

Table 2 Functional group percentage (^{13}C NMR spectra) of sample fractions and reference materials. The ^{13}C NMR ranges used were: I=0-50 ppm, II=50-100 ppm, III=100-160 ppm, IV=160-190 ppm.

CODE	*NMW (KDa)*	*Aliphatic I (%)*	*Carbohydr II (%)*	*Olef+arom III (%)*	*Carboxyl IV (%)*
DC1	>30	47.6	48.3	4.1	-
DC2	10-30	55.9	24.6	11.6	7.8
DC3	1-10	52.8	21.9	11.2	14.1
E1	>30	17.8	47.7	27.1	7.4
E2	10-30	16.7	45.9	26.3	11.0
E3	1-10	16.9	46.4	19.0	17.6
IN1	>30	27.4	38.5	23.7	10.3
IN2	10-30	17.0	46.9	23.7	12.5
IN3	1-10	31.6	35.6	17.6	15.2
OUT1	>30	39.5	32.3	20.8	7.4
OUT2	10-30	27.0	37.4	23.3	12.2
OUT3	1-10	28.0	36.2	20.1	15.7
IND	-	12.5	32.7	42.6	8.4
LIGN	-	16.9	35.2	43.4	4.5
NOFA*	XAD	27.1	32.4	24.4	14.1
NOHA*	XAD	25.5	32.9	30.0	10.2

*Result from ref. 43

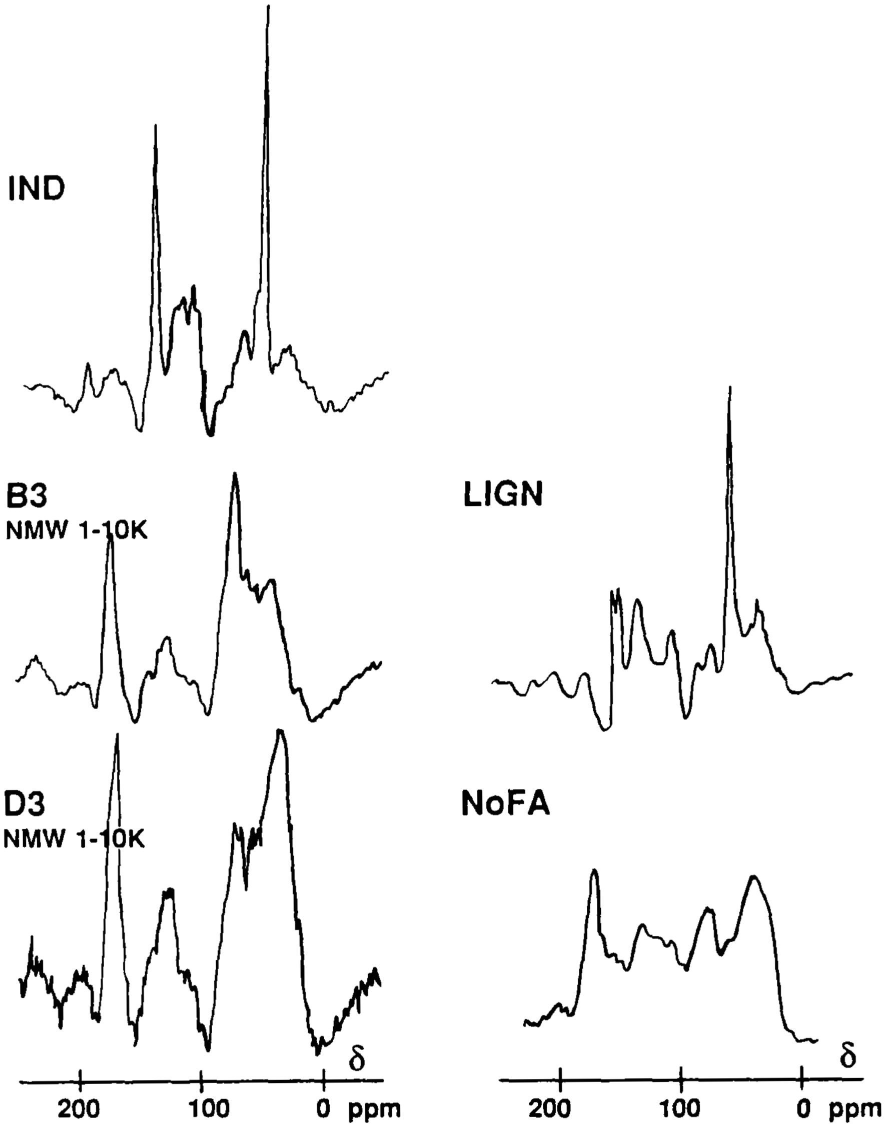

Figure 3 ^{13}C CP/MAS spectra of E3 (E stage), IN3 (inflow), OUT (outflow), IND, LIGN, and NOFA[43] samples.

NMR spectra

Examples of the ^{13}C NMR spectra are given in Figure 3 and results of the integration of the four ranges (regions I-IV) of the ^{13}C NMR spectra of mill sample fractions in Table 2. The resonances of region I (0-50 ppm) are due to aliphatic carbons (methyl, methylene, and

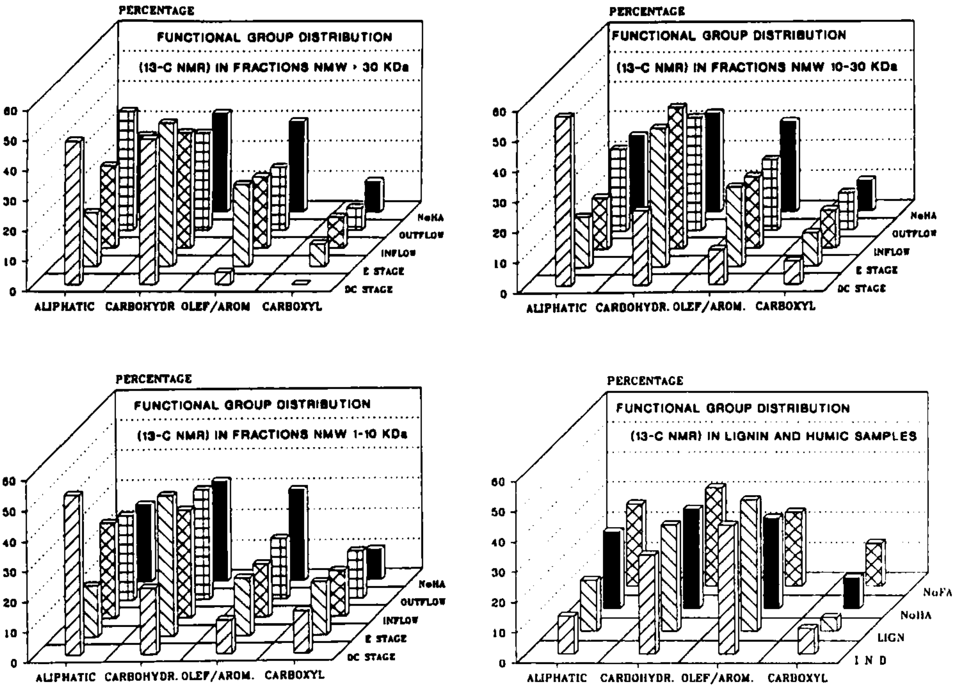

Figure 4 Distributions of functional groups according to ^{13}C CP/MAS NMR of high-molecular chlorolignin fractions and reference materials.

methine) of different origin. The degradation of lignin and the cleavage of aromatic ring of residual lignin during bleaching produce aliphatic structures.[16,29] The resonances of region II (50-100 ppm) arise from carbons bonded to electronegative atoms of oxygen, chlorine, or nitrogen. The chemical shift of methoxyl carbons is about 56 ppm.[3,4] Peaks of carbohydrate carbons of cellulose origin and of the oxygen-bonded carbons of lignin side chains appear between 60 and 100 ppm.[3,5] Region III (100-160 ppm) contains the resonances of olefinic carbons resulting from the cleavage of aromatic rings of lignin in the bleaching process[16] and aromatic carbons of different types. The resonances at the region 100-140 ppm are due to chlorine, alkyl, and nonsubstituted aromatic carbons and/or olefinic carbons, while the resonances at 140-160 ppm are due to oxygen bearing aromatic carbons.[46] These carbons appear as an intense peak in the kraft lignin (IND) spectrum (Figure 3). By contrast, the aromatic carbons bearing oxygen atoms are nearly absent from the chlorolignin samples (E3, IN3 and OUT3, Figure 3). This reflects the degradation of the oxygen-substituted aromatic rings to small molecular compounds (chlorophenols, catechols, and guaiacols[1,47]) or to olefinic chains[16] in bleaching. Region IV (160-190 ppm) contains signals from carboxyl carbons. Signals of the carbonyl carbons of aldehydes and ketones (190-220 ppm) were found only in the spectra of kraft lignin model Indulin ATR (3.8%).

Functional group distribution obtained from ^{13}C spectra of chlorolignin fractions and reference materials are given in Figure 4. Figures 3 and 4 and Table 2 show that the overall

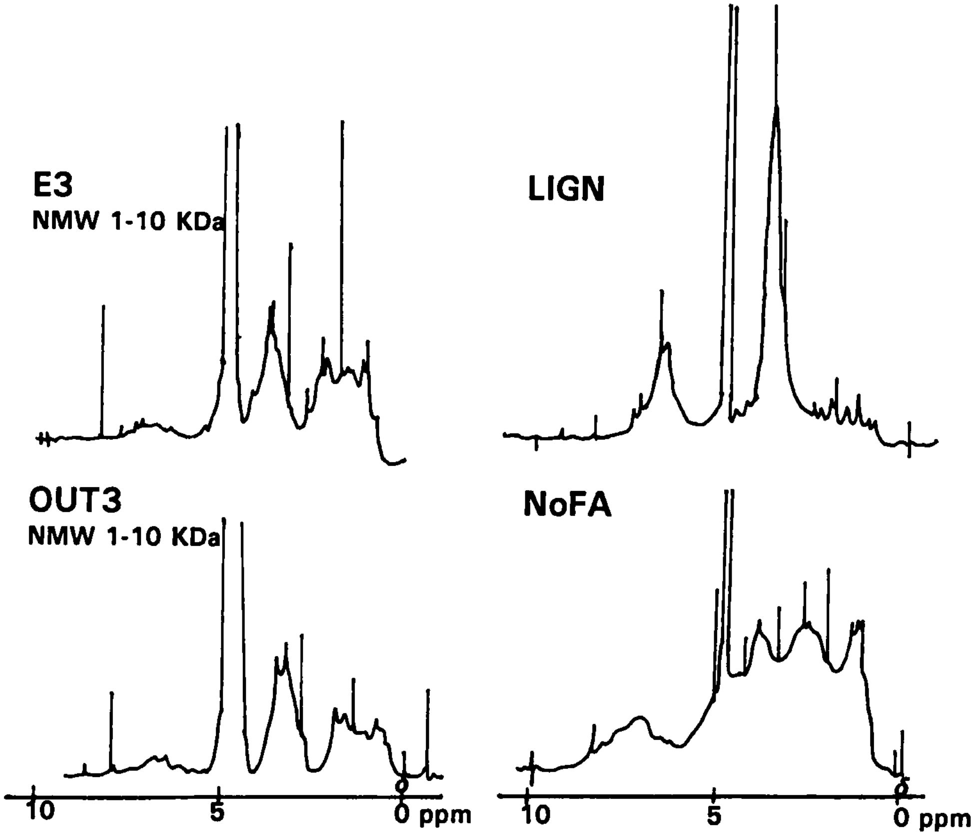

Figure 5 ^{1}H NMR spectra of E3 (E stage), OUT3 (outflow), LIGN and NoFA samples as sodium salts in D_2O.

change in the distribution of carbons from spent bleach liquors (samples DC, E,IN) to pulp mill effluent (sample OUT) is small in all NMW fractions. There are no marked differences in the relative amounts of aromatic + olefinic and carboxyl carbons before (samples E and IN) and after (sample OUT) the biological purification. The portion of aliphatic carbons increases and the portion of carbohydrate carbons slightly decreases from sample E to sample OUT. Sample DC (D/C stage) is clearly more aliphatic and less aromatic than other samples. The differences in the carbon distribution between the NMW fractions of each sample are significant for the carboxyl carbons. In all samples the portion of carboxyls is highest in the lowest of the high molecular weight fractions (NMW 1-10 KDa). The carbohydrate content of sample DC decreases strongly from fraction I (NMW>30 KDa) to fraction II (NMW 10-30 KDa). Comparison of the lignin reference samples (IND and LIGN) with the high-molecular-weight chlorolignins show that latter have lower aromaticity and higher contents of aliphatic and carboxyl carbons. In the spectra of humic reference samples (NoFA and NoHA) the portions of all functional groups are very much the same as in chlorolignin samples IN and OUT.

Table 3 Assignments of signals in the ^{1}H NMR spectra of mill samples and reference materials.

δ value (ppm)	*Assignment*
0.9-1.4	methyl and methylene protons in alkyl group
2.0-2.6	methylene and methine protons α to aromatic ring, carboxyl or carbonyl group
3.4-4.3	protons attached to carbon atom bound to oxygen
6.4-7.5	aromatic and olefinic protons

Figure 5 shows the proton NMR spectra for the mill samples E3 (E-stage, NMW 1-10 KDa) and OUT3 (outflow, NMW 1-10 KDa) measured in deuterated water, and Table 3 gives the assignments of the main proton signals in the ^{1}H NMR spectra of mill sample fractions and reference materials. The chemical shift values always refer to the top of the peak, though the peaks themselves (see Figure 5) are broad and irregular in shape.

Table 4 gives the results of integration of the three ranges (regions I-III) of ^{1}H NMR spectra of mill sample fractions for samples E, IN and OUT and reference samples. All ^{1}H NMR spectra of mill samples and humic substances indicate much greater contents of aliphatic protons and protons attached to carbon atoms bound to oxygen than of aromatic protons. In general, aliphatic groups are rich in hydrogens, whereas substituted aromatic rings carry few, if any, hydrogen atoms. The significantly greater aliphaticity than aromaticity of chlorolignins and humic samples is clear, however, only in the carbon spectra (Table 2 and Figure 3). By contrast, the proton NMR spectra of native and kraft lignin show very small portions of aliphatic protons (region I) and the portion of methoxyl (region II) and

Table 4 Functional group percentage (^{1}H NMR spectra) of sample fractions and reference materials. The ^{1}H NMR ranges used were: I=0.2-2.8 ppm, II=2.8-4.4 ppm, III=6.0-8.0 ppm.

CODE	*NMW (KDa)*	*Aliphatic I (%)*	*Carbohydr II (%)*	*Olef;plarom III (%)*
E1	>30	51.1	36.6	12.3
E2	10-30	56.3	30.2	13.5
E3	1-10	60.3	32.2	7.5
IN1	>30	50.8	38.7	10.5
IN2	10-30	55.0	33.7	11.3
IN3	1-10	64.7	26.7	8.6
OUT1	>30	55.5	39.2	5.3
OUT2	10-30	45.1	46.3	8.6
OUT3	1-10	45.9	45.6	8.5
IND	-	14.0	53.5	32.5
LIGN	-	25.4	55.9	18.7
NOFA	XAD	51.0	38.6	10.4
NOHA	XAD	40.0	43.4	16.6

aromatic protons (region III) dominates. The differences between the proton spectra of mill sample fractions (DC-OUT) are small.

The ^1H and ^{13}C NMR spectra of mill sample fractions together show that lignin undergoes a pronounced change in the bleaching process. There are no marked differences in the structures of the high-molecular-weight fractions before and after the purification plant. Comparison with the NMR spectra of humic materials show that the total pulp mill effluent (sample OUT) resembles the spectra of fulvic acid (NoFA). Table 1 shows that the total amount of organic material (OM) is decreased in the purification process.

IR Spectra

The IR spectra of chlorolignins and humic substances show relatively few absorption bands that are very broad. The simplicity of the spectra is due to the polymeric nature of the materials. Fig. 6 shows some typical examples of the IR spectra.

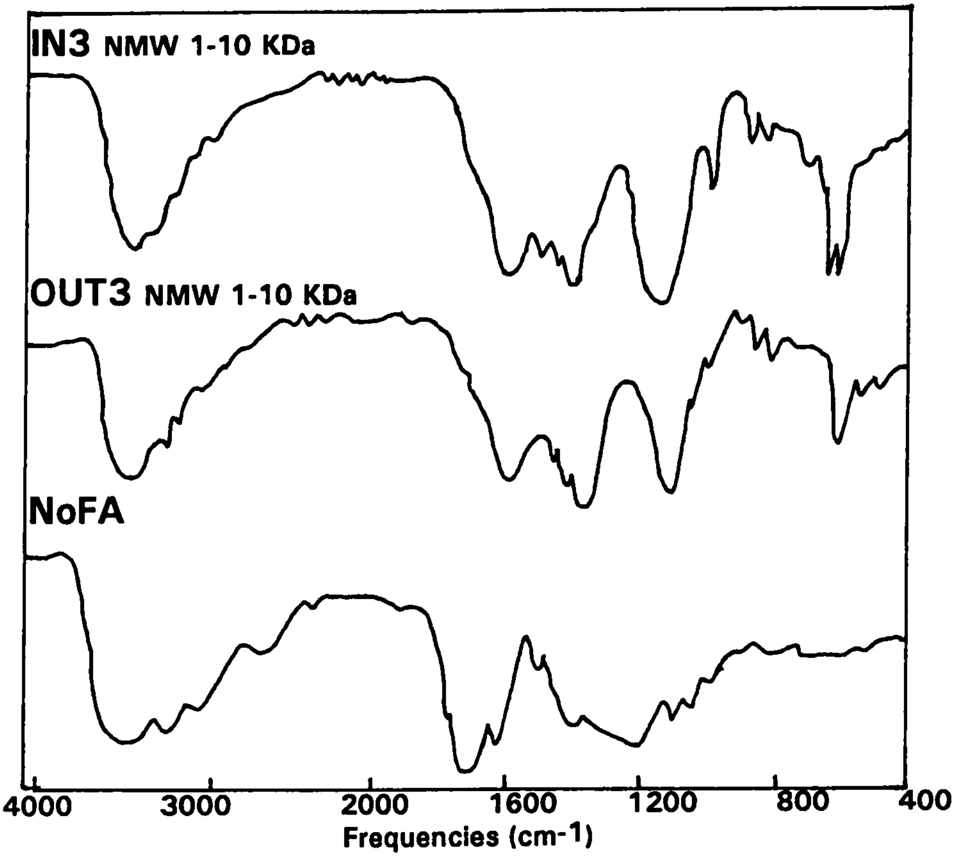

Figure 6 Infrared spectra of sample IN3 (inflow, NMW 1-10 KDa), sample OUT3 (outflow, NMW 1-10 kDa), and reference Nordic fulvic acid (NoFA).

The characteristic absorption bands in the IR spectra of chlorolignin fractions (DC-OUT, 1-3) are similar in all the spectra measured. The spectra were obtained using sodium salts. The absorption band at 3400 cm^{-1} indicates O-H stretching and the broad band at 2900 cm^{-1} C-H stretching of aliphatic methyl and/or methylene units. The strong band at 1600 cm^{-1} and weak band at 1400 cm^{-1} are typical of stretching in carboxylic salts.[17] The strong absorption bands centered at 1140 cm^{-1} arise from C-O stretching vibrations and from C-Cl stretching of aromatic units. In all infrared spectra of chlorolignin fractions there are very clear absorptions at 900-600 cm^{-1} due to the aliphatic C-Cl vibrations. These bands are most intense in the fractions NMW 1-10 KDa. The presence of double bonds cannot be seen as separate bands (1650-1600 cm^{-1}) in the IR spectra.

The reference humic substances NoHA and NoFA were isolated from water using the XAD-8 resin method.[42] The infrared spectra were run using samples in the acid form. The characteristic absorption band of the carbonyl stretching appears at 1730 cm^{-1}, which is typical for the acid form. The strong stretching vibration of the O-H group appears at 3400 cm^{-1}. Bands at 3000-2800 cm^{-1} are due to the C-H stretching of aliphatic units, and C=C double bond vibrations appear at 1630 cm^{-1}. The very broad bands at 1500-1000 cm^{-1} arise from aliphatic C-H bending (1380 cm^{-1}), alcoholic or carboxylic O-H bending, C-O stretching, and/or aromatic C-C stretching vibrations (1300-1000 cm^{-1}).[30,31] C-Cl bands are not observed.

The most significant difference in the IR spectra of the chlorolignin fractions and the reference humic materials is the presence of strong C-Cl absorption bands in the chlorolignin spectra and their absence in the spectra of humic samples. The presence of aliphatic C-Cl bonds is unambiguous in the IR spectra of the chlorolignins but the existence of aromatic C-Cl bonds is uncertain because any C-O vibrations overlap in the region of the strong band at about 1140 cm^{-1}.

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