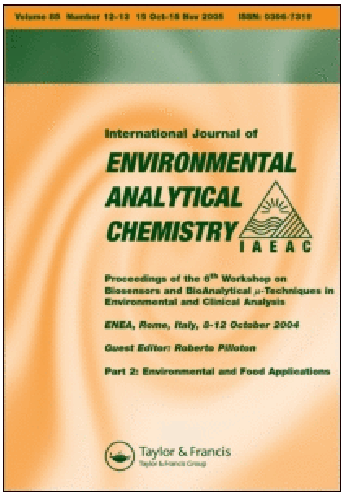

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CHARACTERIZATION OF CHLOROLIGNINS IN BLEACHED KRAFT PULP MILL EFFLUENTS USING ELEMENTAL ANALYSIS AND FINGERPRINTING BY CuO OXIDATION AND HPLC

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Elemental analyses and chemical degradation techniques were applied to characterize lignin compounds in different stages of the chlorine bleaching process and in total mill effluent. Ultrafiltration was used for separation of chlorolignins into three nominal molecular weight fractions. Alkaline CuO oxidation products were analyzed by HPLC with diode array detection. Major compounds were identified by reference to authentic compounds. For comparison, native lignins (MWL), kraft lignins, and humic substances were studied as well. The oxygen, carbon and organic chlorine contents were closely similar in the various mill samples and their fractions. In all mill samples, vanillin and 6-chlorovanillin were the most abundant CuO oxidation products. There were no essential differences between the effluents before and after the activated sludge treatment. The results indicate the stability of chlorolignins during the bleaching and in particular during the purification. The fingerprint CuO/HPLC and elemental analyses showed clear differences between native lignins, humic substances and industrial chlorolignins. The method can be used for following the fate of chlorolignins in waterways and characterizing high-molecular-weight material.

KEY WORDS: Chlorolignin, structural characterization, alkaline CuO-oxidation, reversed-phase HPLC, elemental analysis.

INTRODUCTION

Chlorobleaching of pulp is a significant source of pollution of recipient natural waters. During the bleaching process the residual lignins react with chlorine or other chlorine-containing bleaching agents, producing a complex mixture of organic compounds of various molecular sizes. Approximately 80% of the organic material in the spent bleach liquors of kraft pulp consists of the relatively high-molecular-weight material (MW>1000) known as chlorolignins.[1]

Chlorinated compounds, especially chlorolignins, are only partially removed by wastewater treatment.[2,3] The chemical structures and the chemical and environmental behaviour of chlorolignins are poorly understood. Recent studies have shown chlorolignins to be biologically inactive due to their macromolecular nature,[1] and non-toxic to aqueous organisms.[4] However, biological[5] and chemical[6,7] degradation or sorption/desorption processes[8] make them precursors of harmful low-molecular-weight chlorinated phenolic compounds such as chlorinated catechols, guaiacols, veratroles, and vanillins. These compounds cause sublethal effects, odour and off-taste in the recipient waters.[9,10] Although the use of chlorine chemicals in pulp bleaching will decrease in the future, the need to know more about the chemical structure, harmful effects, and environmental behaviour of chlorinated high-molecular-mass materials continues.

The major part of the dissolved organic matter in natural waters consists of acidic polymers generally classified as humic substances. The combined effects of humic substances and chlorolignins on the aquatic environment are not known.

The residual lignins undergo dramatic structural changes in the bleaching process. Although many comprehensive experiments have been performed to characterize the structures of chlorolignins,[1,4,11–16] the results obtained have not been unambiguous. Structural studies have shown chlorolignins to have high carbonyl and carboxylic contents, while the methoxyl and hydroxyl contents are low.[1,4,11–14,16] The adsorbable organic chlorine (AOX) concentration is 5–10% by weight, depending on process parameters, and seems to be low in the aromatic units. The content of phenolic structures is low and depends on the experimental method used.[17,18]

Ultrafiltration (UF) is commonly used to fractionate samples according to molecular size. Being a relatively inexpensive, expedient, simple, non-destructive, and reagent-free technique, it has been widely used to separate high-molecular-weight chlorolignins from small molecules and inorganic salts. Cut-off values of filters used for chlorolignin isolation are typically 1000 or 2000.[4,6,11–13,16,18–20] UF separations of natural organic polymers, such as humic substances, indicate that the results may depend to varying degree on several physicochemical parameters, including concentration, ionic strength, and pH.[15,21] It has been shown recently[15] that the amount of components in bleached kraft mill effluent retained on UF (MW > 10,000) decreased upon dilution with distilled water indicating that the halogenated components occurred as micellar aggregates.

Metal oxide oxidations have been widely used for degradation of high-molecular-weight materials such as lignins and humic substances. Chlorolignin samples have been oxidized with permanganate,[11–13, 17–18] and humic materials and lignins of different origin[22–29] mostly by milder CuO treatment. Gas chromatography and mass spectrometry (GC-MS) has usually been applied for analysis of the degradation products of lignins and humic substances. Reversed-phase high-performance liquid chromatography (RPHPLC) has been used in identifying the CuO oxidation products of softwood lignins in forest humus layers[30–31] and various plant samples[29,32–34] and also to separate phenols, phenolic aldehydes, ketones, and acids.[35] Pyrolysis combined to GC-MS is a modern analytical technique which has also been applied to characterization of lignin and humic substances.[36,37]

Earlier studies on humus and lignin samples [38,39] have shown CuO oxidation followed by RPHPLC analysis of phenolic degradation products to be a promising procedure to characterize the structural building blocks of chlorolignins. The aim of the present study was to learn more about the chemical character of chlorolignins in successive stages of the chlorobleaching process and in effluent waters before and after activated sludge treatment in purification plants. The reliability of the results was assured by measuring the UV spectra of the HPLC peaks (diode array detector) and comparing these with the spectra of reference compounds synthesized in our laboratory.[40,41] Ultrafiltration was used to isolate and separate high-molecular-weight material from effluent waters but not for exact molecular weight determinations. Three molecular weight (MW) fractions of each sample were analyzed separately in order to characterize the distribution of chlorine, structural units, and functional groups in the fractions of chlorolignins.

EXPERIMENTAL

Reference compounds

Several chlorinated and non-chlorinated reference compounds were used to assist in optimizing the HPLC conditions and in interpreting the chromatograms. Two standard mixtures were prepared. The mixture of non-chlorinated lignin-type phenolics consisted of 13 compounds (see Figure 2A below): 3,4-dihydroxybenzoic acid (**1**), vanillyl (4-hydroxy-3-methoxybenzyl) alcohol (**2**), 4-hydroxybenzoic acid (**3**), vanillic (4-hydroxy-3-methoxybenzoic) acid (**4**), 4-hydroxybenzaldehyde (**5**), veratryl (3,4-dimethoxybenzyl) alcohol (**6**), 4-hydroxyacetophenone (**7**), vanillin (4-hydroxy-3-methoxybenzaldehyde) (**8**), 4-hydroxy-cinnamic acid (**9**), acetovanillone (4-hydroxy-3-methoxyacetophenone) (**10**), veratric (3,4-dimethoxybenzoic) acid (**11**), 2,4-dihydroxyacetophenone (**12**), and veratraldehyde (3,4-dimethoxybenzaldehyde) (**13**). The mixture of chlorinated vanillic acids and vanillins consisted of 12 compounds (see Figure 2B below): 2-chlorovanillic acid (**14**), 6-chlorovanillic acid (**15**), 5-chlorovanillic acid (**16**), 2,5-dichlorovanillic acid (**17**), 5,6-dichlorovanillic acid (**18**), 2-chlorovanillin (**19**), 5-chlorovanillin (**20**), 6-chlorovanillin (**21**), 2,5-dichlorovanillin (**22**), 2,6-dichlorovanillin (**23**), 5,6-dichlorovanillin (**24**), and trichlorovanillin (**25**). Syringic (4-hydroxy-3,5-dimethoxybenzoic) acid (**26**), syringaldehyde (**27**) and acetosyringone (4-hydroxy-3,5-methoxyacetophenone)(**28**) were also used as reference compounds. Several other compounds known as precursors or degradation products of lignin polymers were tested for the interpretation of minor chromatographic signals. The chlorinated reference compounds were prepared in our laboratory[40,41] and the other non-chlorinated reference compounds were commercially available.

Samples and sample preparation

Samples. Samples were obtained from a kraft softwood pulp mill in Central Finland (Äänekoski Mills) employing a five-stage (D+C)EDED bleaching sequence (D+C = chlorine dioxide + chlorine treatment, E = alkaline extraction, and D = chlorine dioxide

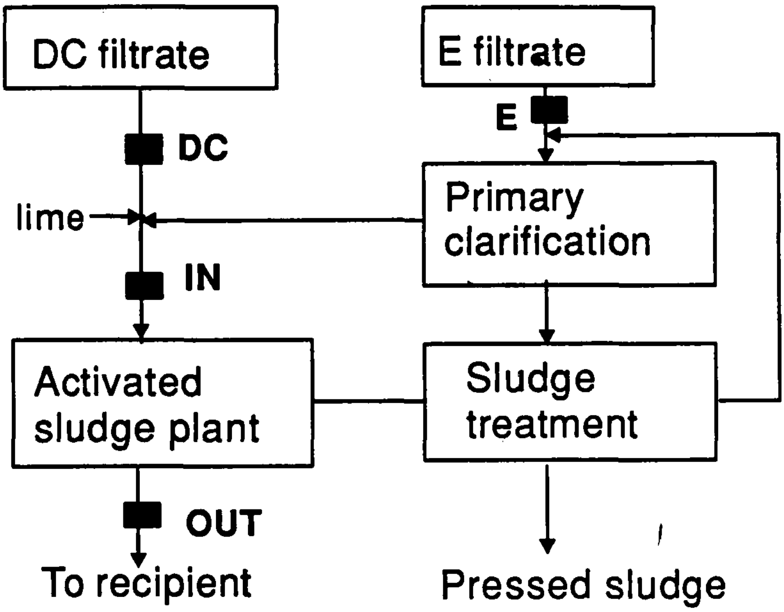

Figure 1 Effluent treatment and sampling points at the pulp mill; DC = chlorine dioxide + chlorine, E = alkaline treatment, IN = inflow to purification plant, and OUT = outflow to recipient water.

treatment) (kappa number 25–27) and an activated sludge treatment for the total effluent. Effluents were sampled at four points: the (D+C) stage (sample DC), the E stage (sample E), and the inflow (sample IN) and outflow (sample OUT) streams of the biological purification plant (activated sludge) (Figure 1).

The effluent waters were centrifuged and filtered through a membrane filter (0.45μm). The pH of the water samples was adjusted to 10.0 to prevent the formation of hydrogen bridges and aggregation during the ultrafiltration procedure. The following lignin and humic preparations were used as reference materials: MWL lignin (pine) (sample LIGN), commercial kraft pine lignin (Indulin AT-R, Westvaco Co.) (sample IND) and Nordic fulvic acid reference (sample NOFA) from Norwegian humic lake Hellerudmyra.

Ultrafiltration (UF). One-liter samples (DC, E, IN, and OUT) were ultrafiltered using a tangential flow membrane system (Millipore) with three molecular weight cut-off filters (PTTK, PTGC and PCAC). The high-molecular-weight fractions (1, MW > 30,000; 2, MW 10,000–30,000 and 3, MW 1,000–10,000) obtained for each sample (DC, E, IN, and OUT) were freeze-dried and the residual masses weighed. The samples were stored frozen for further analysis. Organic matter (OM) contents were determined by subtraction of ash from the weighed masses of ultrafiltration.

Elemental analyses and TOCl determination

Elemental analyses (C, N, and H) were carried out by various combustion techniques with a Carlo Erba Elemental Analyzer at the University of Joensuu. Total amount of organically bound chlorine (TOCl) was determined by the oxygen combustion method.[42] The residual weight of ashless samples were assumed to be the summed content of oxygen and sulfur.

CuO oxidation and solid-phase extraction (SPE) column purification

Alkaline cupric oxide oxidation was carried out in a homemade pressure bomb using a modified version of the method described by Kögel and Bochter.[30] 10-mg amounts of freeze-dried brownish powder of a mill sample fraction (DC—OUT, fractions 1–3) and of each reference material (LIGN, IND, NOFA) were placed in a pressure bomb. 50 mg CuO, 10 mg $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, and 3 ml 2 M NaOH were added and the vessel was sealed under nitrogen. The bomb was maintained at 170°C reaction temperature for 2 hours after which the suspension of degradation products was cooled, centrifuged, washed, and acidified.[30] The phenolic degradation products in the clear centrifuged solution (pH 2.0) were concentrated and purified on disposable sorbent extraction (SPE) columns. The SPE column material was reversed-phase C-18 with particle size 40μm and filling volume 3 ml (Baker, Phillipsburg, USA). Other SPE materials tested for the purification were C-8, phenyl, and high hydrophobic C-18 HHP (Baker). However, the recoveries of a standard mixture of four reference compounds (vanillic acid, vanillin, 5-chlorovanillin, and 5,6-dichlorovanillin) were poorer on these materials than on C-18 SPE columns, in which case a recovery of 81–90% was achieved. Degradation products were selectively eluted from the SPE column with ethyl acetate, the ethyl acetate was removed with nitrogen, and the residue was dissolved in 1 ml of methanol (Rathburn). Samples were kept in the dark and refrigerated (+4°C) prior to HPLC analysis. When control CuO oxidation experiments were performed on mono-and dichlorinated vanillins, the chromatograms of degradation products indicated that the main part of the starting materials remained undegraded. The oxidation products comprised non-chlorinated and monochlorinated vanillins and vanillic acids.

Liquid chromatography

HPLC was carried out on a Model 1090 liquid chromatograph (Hewlett-Packard) equipped with an automated variable-volume injector and a diode array UV-visible spectrum detector, both controlled by HP ChemStation (Hewlett-Packard). The reversed-phase column was Spherisorb ODS, 5 μm, 250 × 4.6 mm (HPLC-Tech), and a (10 × 2 mm) precolumn (Vydac, 40 μm) was used. All analyses were run with linear gradient elution, with 0.01 M phosphate buffer, pH 2.0 and acetonitrile (Rathburn). The starting eluent contained 10% acetonitrile and 90% phosphate buffer and the gradient was terminated after 50 min when the concentration of acetonitrile had reached 50%. During an additional 10 min, the concentration of acetonitrile increased to 70%. The injection volume was 20 μl. Chromatograms were recorded at 280 nm. The UV spectra were determined for integrated peaks of the chromatograms. For identification purposes, the retention times and UV spectra of sample peaks were compared with those of reference compounds.

RESULTS AND DISCUSSION

Elemental analysis and organic matter (OM)

Table 1 shows the sample data, the OM and ash contents, organic chlorine contents and elemental ratios H/C, O/C and C/Cl of samples and reference materials. The atomic ratio of oxygen to carbon (O/C) shows clearly that the mill samples (DC, E, IN, and OUT) are strongly oxidized compared to reference materials MWL lignins (LIGN), kraft lignins (IND), and humic sample (NOFA). This is a similar finding to those of Bennett *et al.*[14] and Mörck *et al.*[18] The oxygen and carbon concentrations were closely similar in all mill samples, and there were no marked differences between the various ultrafiltrated fractions of each sample. On the other hand, the carbon concentrations are higher and O/C ratios lower in the reference materials compared to those of the mill samples. The atomic ratio of hydrogen to carbon (H/C) is related to the per cent saturation of carbon atoms. H/C values in mill samples vary from 1.2 to 1.6 and show significant aliphatic nature in all fractions. The present result is in good agreement with the earlier studies with ^{13}C-NMR spectroscopy.[16]

Table 1 Organic matter (OM), ash, organic chlorine contents and atomic ratios of NMW fractions and reference materials.*

Sample	*NMW*	*OM*		*ΣOM*	*Ash*	*H/C*	*O/C*	*C/Cl*	*Cl*
code	*(K)*	*(mg/l)*	*(%)*	*(mg/l)*	*(%)*				*(%)*
DC1	>30	40.6	(12.4)		19.8	1.55	1.01	18.6	6.1
DC2	10–30	84.7	(25.8)		9.4	1.29	0.90	15.1	7.8
DC3	1–10	202.7	(61.8)	328.0	12.8	1.32	0.98	15.1	7.3
E1	>30	806.5	(41.1)		8.7	1.18	0.72	23.8	5.7
E2	10–30	794.9	(40.5)		13.4	1.18	0.84	25.5	5.0
E3	1–10	361.9	(18.4)	1963.3	18.9	1.18	0.76	24.9	5.3
IN1	>30	212.1	(32.3)		11.5	1.34	0.76	26.1	5.1
IN2	10–30	235.4	(35.8)		10.9	1.25	0.85	23.4	5.4
IN3	1–10	209.9	(31.9)	657.4	15.3	1.23	0.83	27.6	4.7
OUT1	>30	148.5	(33.0)		11.8	1.31	0.76	31.2	4.4
OUT2	10–30	170.3	(37.9)		13.0	1.30	0.85	27.2	4.7
OUT3	1–10	131.0	(29.1)	449.8	16.0	1.28	0.91	31.3	3.8
LIGN	-	-		-	ND	1.22	0.45	-	<0.1
IND	-	-		-	0.6	1.08	0.36	-	<0.1
NOFA	-	-		-	ND	0.92	0.70	-	<0.1

*Percentages are given on ash-free weight basis. ND = not detected; detection limit for chlorine measurements was

The C/Cl ratios were slightly lower in the first chlorination (DC stage) waste than in other stages and slightly higher in outflow samples (OUT) and independent of molecular size. These results are in good agreement with those of other studies of modern kraft pulp mill effluents.[4,16,18] It is a remarkable finding that activated sludge treatment appeared to have no marked influence on the OCI concentration or on the elemental composition in the fractions studied. Only a small decrease was observed for OCI values of chlorolignins after the purification process. Nitrogen concentrations of the mill samples were from 0.2 to 0.6 % and do not need specific discussion. Organic chlorine concentrations (OCI) of reference materials and nitrogen concentration of MWL sample (LIGN) were below the limit of determination (<0.1% for OCI and <0.2% for N).

As shown in Table 1, the amounts of OM in chlorolignin fractions indicate that the average size of the heterogeneous macromolecules was significantly smaller in the DC than in the E stage. This is a consequence of the low pH in the DC and high pH in the E stage. The formation of phenolate and carboxylate anion groups in chlorolignins under alkaline extraction conditions makes the higher-molecular-weight fraction more water-soluble in the E stage. Table 1 also shows that the total amount of OM decreased slightly in the purification process, but the distribution of OM among the molecular weight fractions remained almost the same from E stage to outflow sample (E-OUT).

CuO oxidation and RPHPLC analysis

The alkaline CuO oxidation used for the degradation of high-molecular-weight chlorolignin fractions cleaves the alkyl side-chains of the heteropolymeric material to single carboxyl substituents. Aromatic structures remain untouched and the CuO oxidation yields phenolic aldehydes, ketones and acids.[25] The simple and rapid RPHPLC method has been used for analysis of oxidation products,[30,35] because it does not require complicated purification procedures or derivatization prior to chromatographic analysis. The separations of a mixture of 13 non-chlorinated phenolic compounds and a mixture of 12 chlorinated phenolic model compounds are shown in Figure 2 (UV detection 280 nm). Most of the compounds were well resolved in a single run. Table 2 gives the capacity factors, k′, of the reference compounds; compounds which were identified in the samples are marked with crosses.

Figure 3 shows the HPLC chromatograms of the samples DC, E, IN, and OUT (fraction 3, MW 1–10 K). All the chromatograms of other fractions (fraction 1, MW > 30 K and fraction 2, MW 10–30 K) of mill samples were identical and therefore their chromatograms are not presented. The significant result is the overall similarity between the chromatograms of the different bleaching stages and total mill effluent. The characteristic peak in all chromatograms of the mill samples was that due to 6-chlorovanillin (peak no. **21**, retention time (t_R 29.6 min). Small traces of 6-chlorovanillic acid (t_R 17.0 min) and 2-chlorovanillin (t_R 26.5 min) were found in the effluent samples of chlorine bleaching (DC1-DC3) and alkaline extraction (E1-E2) stages. The main non-chlorinated compounds identified in the chromatograms were vanillic acid (**4**), 4-hydroxybenzaldehyde (**5**), 4-hydroxyacetophenone (**7**), vanillin (**8**), and acetovanillone (**10**).

The presence of 6-chlorovanillin (**21**), the major chlorinated degradation product, indicates the low degree of chlorination in chlorolignins; only non-chlorinated and mono-chlorinated

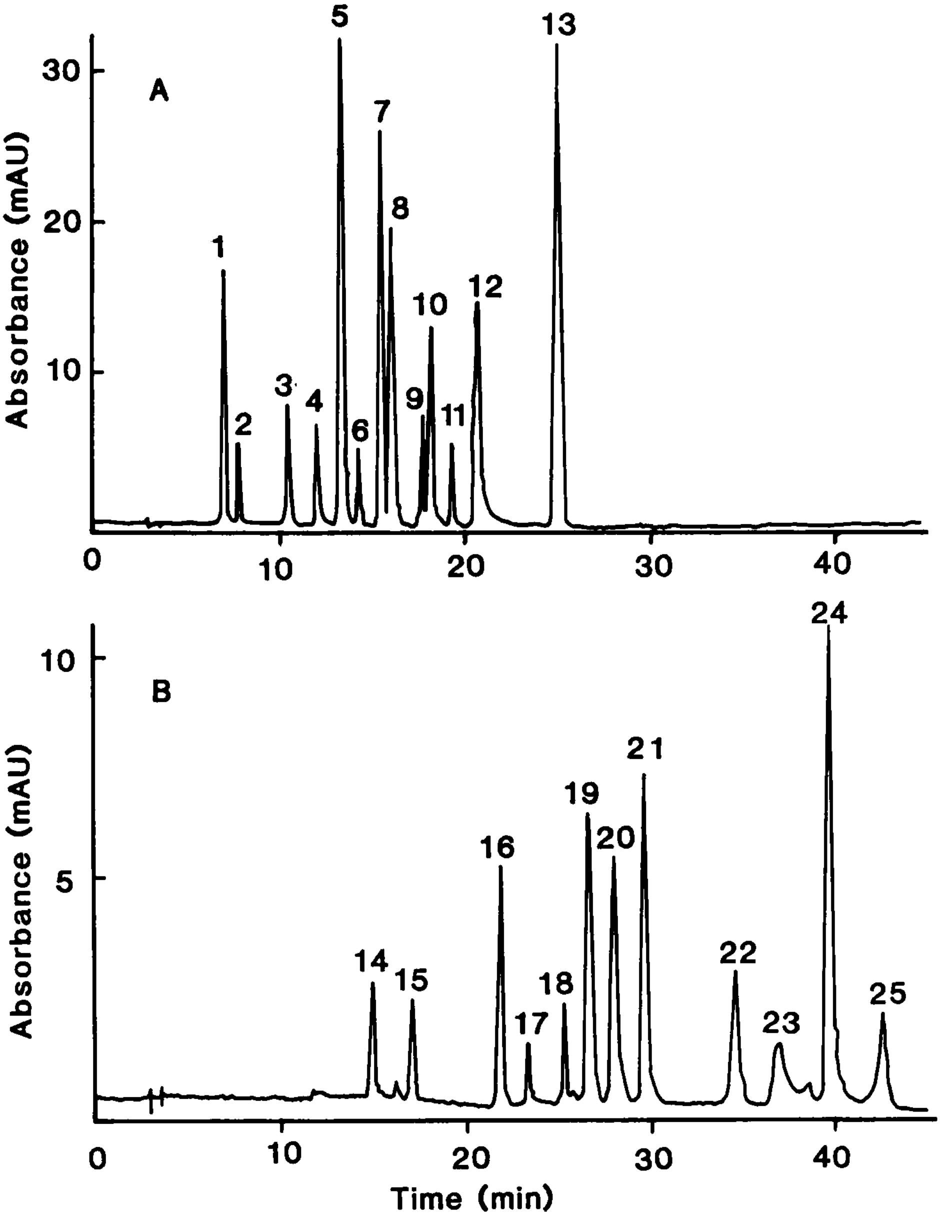

Figure 2 HPLC chromatograms of reference mixtures. (A) Non-chlorinated phenolic compounds, (B) chlorinated vanillic acid and vanillin compounds. For peak numbers, see Experimental.

Table 2 Capacity factors (k′) of reference compounds.*

COMPOUND	*No.*	*k′*	*DC*	*E*	*IN*	*OUT*	*LIGN*	*IND*	*NOFA*
3,4-Dihydroxybenzoic acid	1	1.61	—	—	—	—	—	—	—
Vanillyl alcohol	2	1.91	—	—	—	—	—	—	—
4-Hydroxybenzoic acid	3	2.87	—	—	—	—	—	—	x
Vanillic acid	4	3.50	x	x	x	x	x	x	x
4-Hydroxybenzaldehyde	5	4.00	x	x	x	x	—	x	—
Veratryl alcohol	6	4.32	—	—	—	—	—	—	—
4-Hydroxyacetophenone	7	4.77	x	—	x	x	—	—	—
Vanillin	8	5.00	x	x	x	x	x	x	x
4-Hydroxycinnamic acid	9	5.58	—	—	—	—	—	—	—
Acetovanillone	10	5.79	x	x	x	x	x	x	—
Veratric acid	11	6.23	—	—	—	—	—	—	—
2,4-Dihydr.acetophenone	12	6.74	—	—	—	—	—	—	—
Veratraldehyde	13	8.32	—	—	—	—	—	—	—
2-chlorovanillic acid	14	4.56	—	—	—	—	—	—	—
6-chlorovanillic acid	15	5.33	x	x	—	—	—	—	—
5-chlorovanillic acid	16	7.07	—	—	—	—	—	—	—
2,5-dichlorovanillic acid	17	7.72	—	—	—	—	—	—	—
5,6-dichlorovanillic acid	18	8.41	—	—	—	—	—	—	—
2-chlorovanillin	19	8.82	x	x	—	—	—	—	—
5-chlorovanillin	20	9.32	—	—	—	—	—	—	—
6-chlorovanillin	21	9.95	x	x	x	x	—	—	—
2,5-dichlorovanillin	22	11.8	—	—	—	—	—	—	—
2,6-dichlorovanillin	23	12.6	—	—	—	—	—	—	—
5,6-dichlorovanillin	24	13.6	—	—	—	—	—	—	—
trichlorovanillin	25	14.8	—	—	—	—	—	—	—
syringic acid	26	3.85	—	—	—	—	—	—	x
syringaldehyde	27	5.63	—	—	—	—	—	—	x
Acetosyringone	28	6.67	—	—	—	—	—	—	x

*The compounds identified in mill samples (DC, E, IN, OUT) and reference materials (LIGN, IND, NOFA) are marked with a cross.

compounds were found among the degradation products. This observation is in accordance with the results of Mörck *et al.*[18] Among the degradation products identified by RPHPLC, those derived from guaiacyl units (peaks **4**, **8**, **10**, and **21**) dominated strongly, as earlier observed by Mörck *et al.*[18] Van Buren and Dence[43] have reported the formation of 5-chloro-, 6-chloro-, and 5,6-dichlorovanillins in chlorine-bleached pine kraft pulp and Vander Linden *et al.*[44] the presence of 6-chlorovanillin among the oxidation products of chlorobleached spruce lignins. These and other results[39,45] indicate that in the chlorobleaching of pulp, the electrophilic aromatic substitution by chlorine occurs mostly in the 6 position (para position with respect to the methoxyl group) of the 4-hydroxy-3-methoxy-phenylpropane (guaiacyl) building block of softwood residual lignins. This structural unit yields 6-chlorovanillin in oxidative degradation and seems to be more stable than other units in both strong degradative bleaching conditions and activated sludge treatment. O'Connor and Voss[8] have shown that chlorinated phenolics can become adsorbed onto chlorolignins and be slowly released over time by simple desorbtion process. The present results show that 6-chlorovanillin can be found also in the CuO oxidation products of chlorolignin fractions after the biological

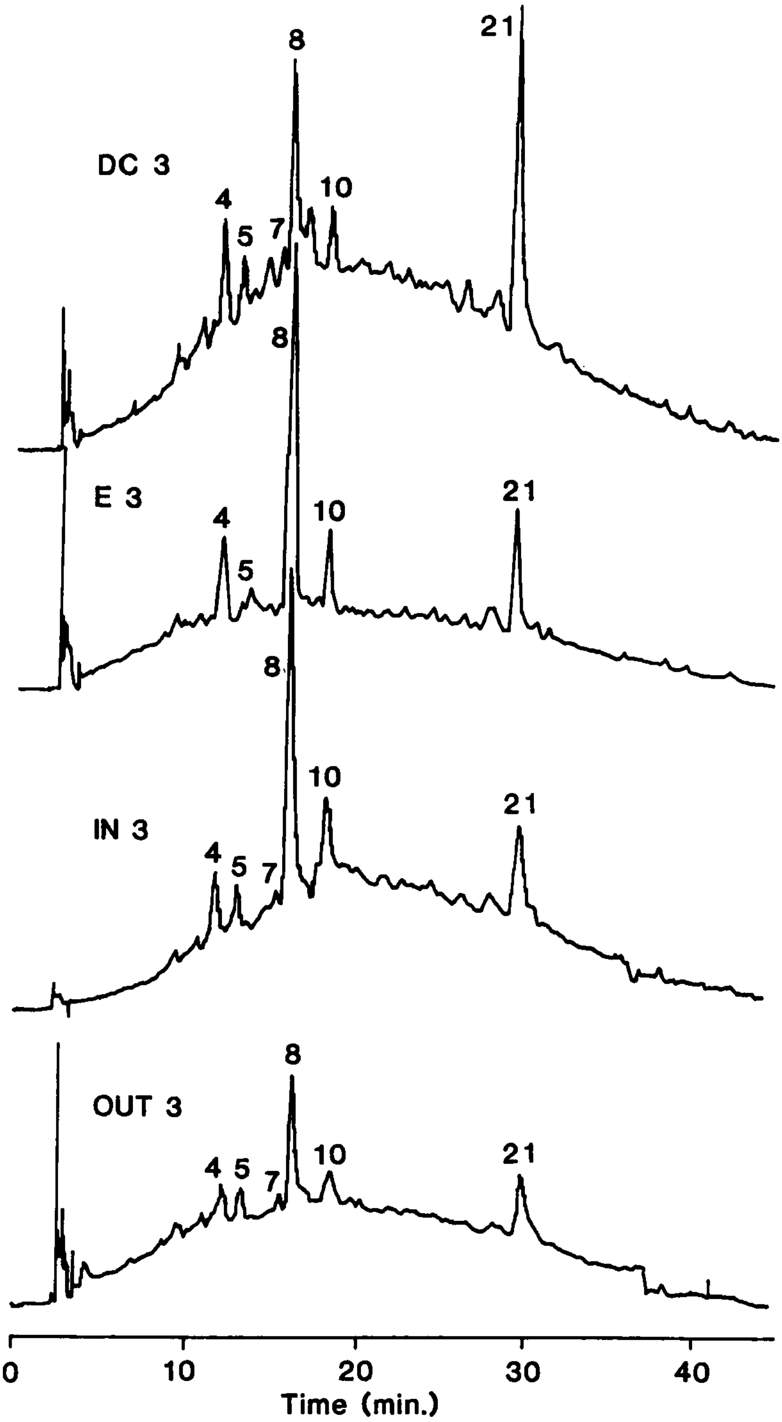

Figure 3 HPLC chromatograms of phenolic compounds from alkaline CuO oxidation of mill samples **DC3** (DC stage), **E3** (E stage), **IN3** (inflow) and **OUT3** (outflow), NMW 1-10 K. For peak numbers, see Figure 2 and Experimental.

purification process and in the products of dissolved chlorolignins in receiving water even 10 km downstream of the pulp mill.[39]

Figure 4 shows the chromatograms of reference samples LIGN, IND and NOFA. The main degradation products of pine MWL lignins (LIGN) were vanillic acid (**4**), vanillin (**8**), and acetovanillone (**10**), with an unknown peak at 18.8 min and with vanillic acid being the main peak. The CuO oxidation of Indulin (IND) yielded the same pattern of non-chlorinated phenolic degradation products as the mill samples. The main peaks of the chromatograms were vanillic acid (**4**), 4-hydroxybenzaldehyde (**5**), 4-hydroxyacetophenone (**7**), vanillin (**8**), and acetovanillone (**10**), with vanillin being the main product. In the chromatogram of the fulvic acid sample (NOFA), the pattern of phenolic degradation products contained almost equal amounts of 4-hydroxybenzoic acid (**3**), vanillic acid (**4**), syringic acid (**26**), vanillin (**8**), syringaldehyde (**27**) and acetosyringone (**28**) as main products. As expected, no chlorinated degradation products were detected for LIGN, IND and NOFA samples. The present results of reference materials MWL lignins, kraft lignins and natural fulvic acid show clear difference in fingerprint chromatograms. The main degradation products in lignin samples are similar, but the ratios of vanillic acid (**4**), vanillin (**8**) and acetovanillone (**10**) are different. The chromatogram of kraft lignins (IND) resembles the chromatograms of mill samples (Figure 3). Among the degradation products of humic sample (NOFA) vanillic acid and vanillin are common to lignin samples and originate from the guaiacyl unit. 4-Hydroxybenzoic acid and syringyl compounds originate from other structural units of humic material.

A comparison of the chromatograms in Figure 3 reveals an overall similarity between all mill samples. The main phenolic components were closely similar not only in all samples but also in all MW fractions of each sample (DC-OUT, 1–3). In earlier investigations of the characteristic structures of chlorolignins as determined by ^{13}C-NMR,[16] we found no marked differences in the structures of the high-molecular-weight fractions of lignins from the E stage (sample E) to total mill effluent (sample OUT). The ^{13}C-NMR spectra demonstrated that the chlorolignins contain olefinic/aromatic structures and carbons adjacent to oxygen or chlorine. Our results obtained by CuO oxidation followed by HPLC of the degradation products confirm that the chlorolignins contain phenolic material, which is partially chlorinated. However, the degree of chlorination of the phenolic structures seems to be low.

The major and minor peaks of the chromatograms were identified by comparison with the retention times and UV spectra of reference compounds. Phenolic aldehydes, ketones, and acids with different substitution patterns in the benzene ring give characteristic UV spectra; different substituents introduce specific features into the spectra. Figure 5 shows the UV spectra of the reference compounds vanillin (t_R 16.0 min) and 6-chlorovanillin (t_R 29.6 min) and of peaks at the same retention times in the chromatogram of sample D3. The HPLC system resolved the phenolic compounds effectively. Through the combination of retention times and UV spectra, the compounds in the chromatograms of the samples were identified reliably.

CuO oxidation followed by RPHPLC analysis of the degradation products has been applied by our group to study the transport of chlorolignins in receiving waters. Earlier chromatograms[39] and those of the present study (Figure 3) indicate that the structure of chlorolignins in the mill effluents and receiving waters is very similar and partially aromatic.

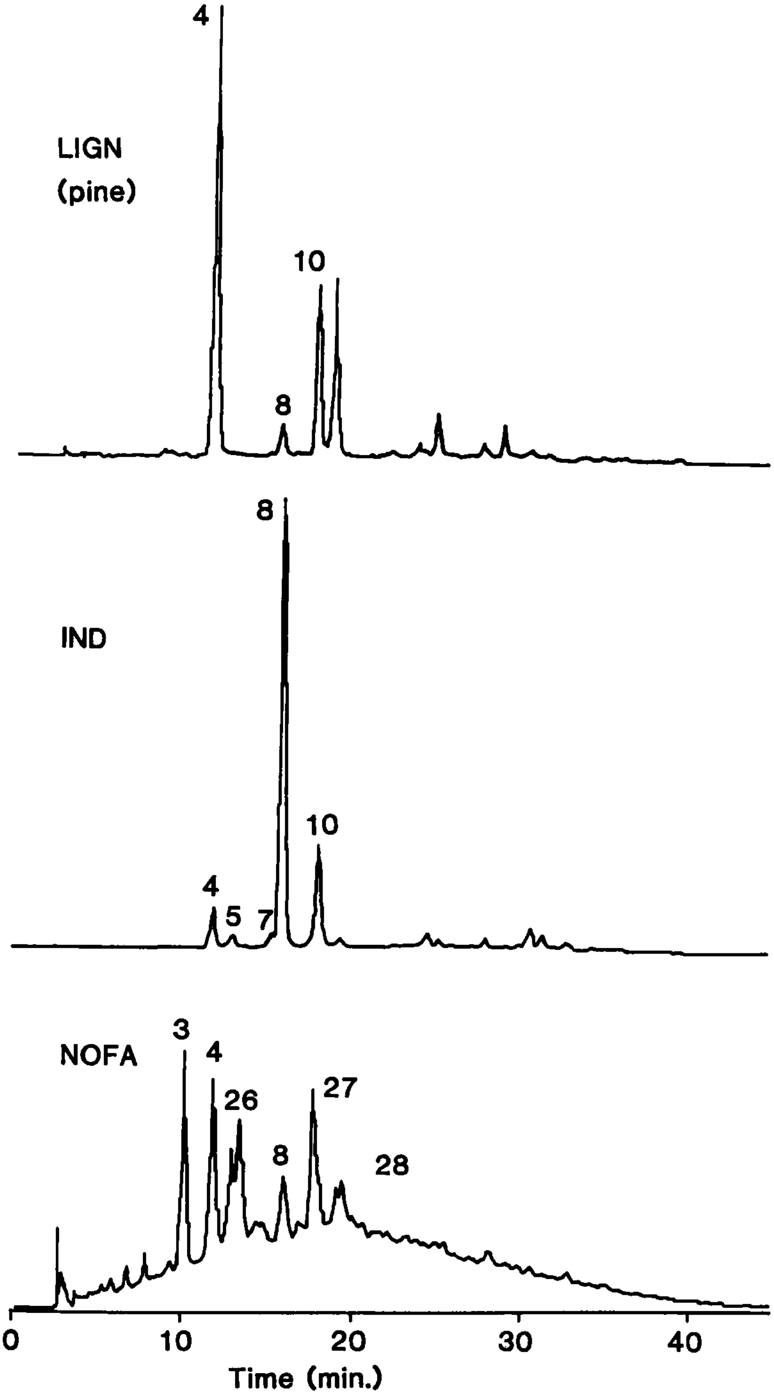

Figure 4 HPLC chromatograms of phenolic compounds from alkaline CuO oxidation of reference samples LIGN (MWL, lignin, pine), IND (kraft lignin), and NOFA (fulvic acid). For peak numbers, see Figure 2 and Experimental.

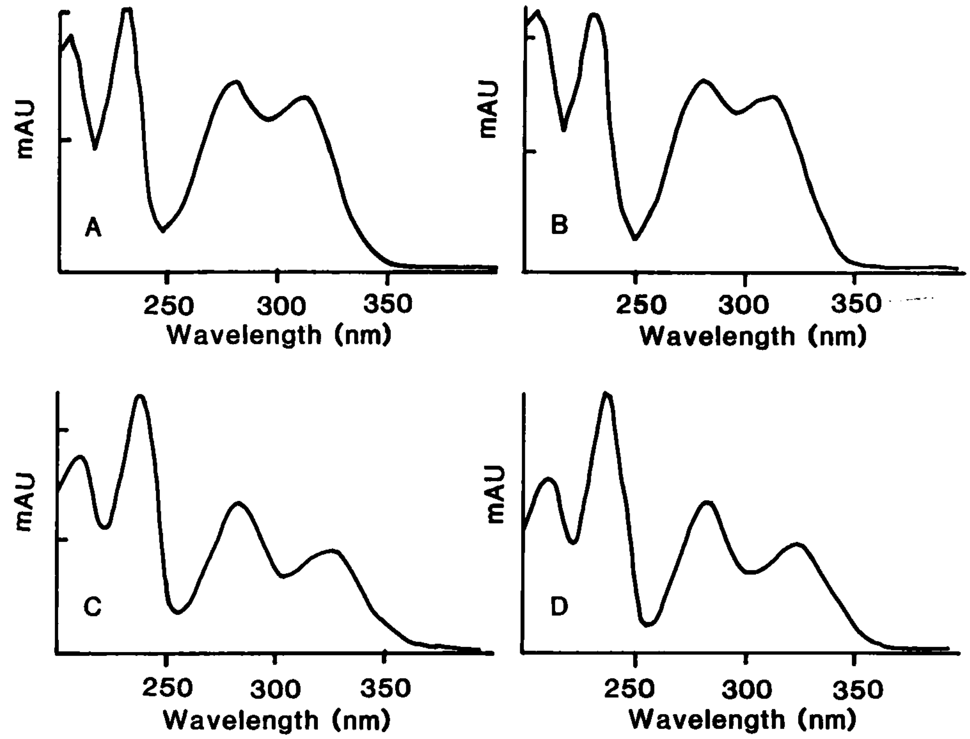

Figure 5 UV spectra of two main peaks of the RPHPLC chromatogram of mill sample DC3 occurring at 16.2 min (**A**) and 29.6 min (**C**) and UV spectra of authentic compounds vanillin (**B**) and 6-chlorovanillin (**D**).

This work confirms that the characteristic phenolic oxidation products (vanillin and 6-chlorovanillin) of high-molecular-weight material are formed in bleaching process and do not change in activated sludge treatment and recipient watercourse. No marked change in the structure of chlorolignins occurred in natural waters downstream of the pulp mill and the same aromatic components were present in the degradation products of high-molecular-weight material as much as ten kilometers below the mill[39]. The contents of vanillin in the samples of oxidated natural humic material and native MWL lignins are low compared to industrial lignins sample (IND) and mill samples (DC-OUT). Other phenolic components than vanillin dominate the chromatograms (measured at 280 nm) of degradated humic matter and native lignin samples. Evidently the aromatic character of water-soluble chlorolignins is stable. In conclusion, vanillin and 6-chlorovanillin can be used as a tracer compounds when following the fate of chlorolignins in water and also in sediment.

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