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CHARACTERIZATION OF HIGH MOLECULAR MASS FRACTIONS OF RECEIVING WATERS AND SEDIMENTS OF A PULP MILL BY CuO-OXIDATION AND HPLC[1]

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ABSTRACT

High molecular mass fractions were separated by ultrafiltration from water samples collected at four sampling stations downstream and one upstream of a kraft pulp mill. These fractions and three sediment samples containing high molecular mass organic compounds were degraded by alkaline cupric oxide oxidation. Lignin and humic compounds in fractions were characterized analyzing their oxidation products by reversed phase HPLC and photodiode array detection.

INTRODUCTION

Cupric oxide oxidation has been widely applied to the characterization of high molecular mass materials. It has been used to study coal formation,[1] lignins from various plants,[2-6] chlorolignins,[7-13] lignosulfonic acids,[14] humic and fulvic acid fractions separated from peat[15,16] and from other humic materials,[17-23] peat as such,[24] sediments,[25-29] bitumen separated from composted materials[30] and even drilling fluid constituents.[31] Few reports have appeared on the cupric oxide oxidation of high molecular mass compounds separated from water samples[32-34] or on the analysis of oxidation products using HPLC.[23,35] Other methods used to characterize the chlorolignins separated from pulp mill effluents include pyrolysis-gas chromatography,[36] IR and NMR,[37-39] gel permeation chromatography[40,41] and potassium permanganate oxidation followed by GC-MS.[42-44] Some of these methods have also been applied to the characterization of chlorolignins in sediments.[45,46] The aim of this study was to test the applicability of CuO oxidation-HPLC method to follow the transport of chlorolignins in the receiving surface waters of a kraft pulp mill. Preliminary results of characterizing chlorolignins in sediments are also given.

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[1] CHEMICAL DEGRADATION PRODUCTS OF LIGNIN AND HUMIC SUBSTANCES. PART III.

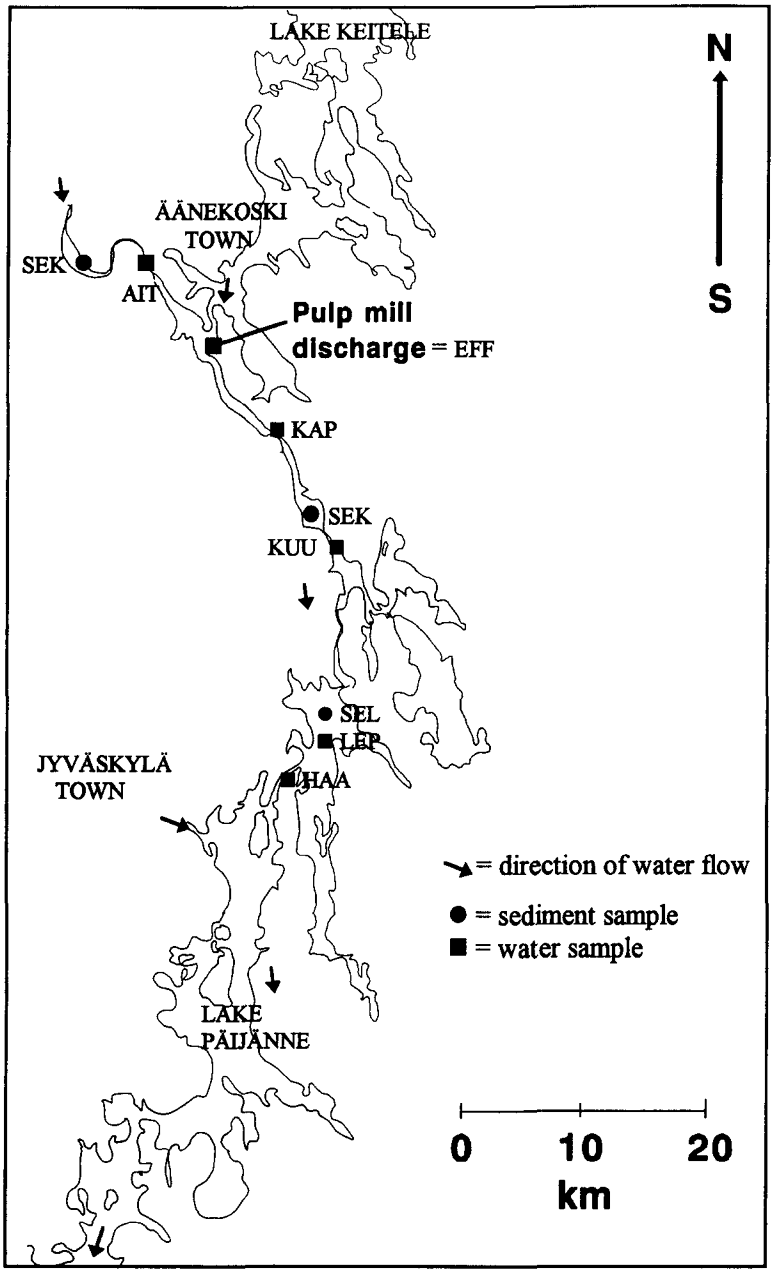


Figure 1. Map of the sampling area and the sampling stations

EXPERIMENTAL

Samples

Six surface water samples and three sediment samples were analyzed. One of the samples was from a brown-coloured (Pt colour about 30) natural humic water upstream of the pulp mill (AIT), one was from the discharged effluent (EFF) and four water samples (KAP, KUU, HAA and LEP) were collected at different distances downstream of the kraft pulp mill using bleaching with chlorine dioxide and activated sludge tratment of the wastewaters. In addition, three surface sediment samples (at each point several samples were taken at 0-5 cm depth from the bottom and mixed), one from an unpolluted area (SEK) and two downstream of the mill (SEV and SEL) were analyzed. Samples were collected in March 1993, except SEV in March 1994. Sampling area and the sampling stations are shown in Figure 1. Later in this text the names mentioned in brackets will be used to refer to oxidation products of the samples in question.

Ultrafiltration and freeze drying

The water samples were adjusted to pH 10 with 0.1 M NaOH and filtrated through a 0.45 μm membrane (Schleicher & Schuell ME 25/21 ST, diameter 47 mm) to remove all macroscopic particles. The samples were ultrafiltrated using a Waters system with two membranes having cutoff-values 1,000 and 10,000 D. The ultrafiltrated fractions were washed twice by adding 200 ml of ultrapure water and continuing ultrafiltration to a volume as small as possible. After freeze drying the fractions obtained with different membranes were weighed, combined and homogenized. The sediment samples were freeze dried as such.

Cupric oxide oxidation

After homogenization, 10 mg the ultrafiltered sample or 100 mg of sediment was weighed and placed into a teflon coated bomb with cupric oxide, iron ammonium sulphate hexahydrate and 2 M NaOH according to Hedges and Ertel.[5] The amounts of these reagents used in the oxidation of ultrafiltrated fractions were 50 mg, 10 mg and 3 ml and for sediments 100 mg, 25 mg and 6 ml, respectively. The bomb was kept for two hours at 170 °C and allowed to cool to ambient temperature. The contents of the bomb were transferred to small test tubes and centrifuged to separate insoluble compounds. Remaining black mass was washed with water and centrifuged again. The combined solutions were acidified with 6 M HCl and allowed to stand for one hour. If a precipitate was observed, the sample was centrifuged again to remove it. The acidic liquid was passed through a Waters Sep-Pak C_{18} microcolumn activated successively with 6 ml of methanol and 6 ml of water. The microcolumn was dried in a flow of nitrogen and eluted with 2 ml of ethyl acetate. This solvent was removed with a gentle flow of nitrogen, the residue was dissolved in 1 ml of methanol and the solution obtained transferred to a vial from which the injection was done. Samples containing materials insoluble in methanol were centrifuged once more before injection.

Liquid chromatography

The CuO oxidation products were analyzed using a HPLC-system consisting of two Waters 501 pumps, a Waters 996 photodiode array detector, a Waters pump control module, a Waters 717 autosampler and a Waters column oven attached to a temperature control module. A 25 cm x 4.6 mm Spherisorb ODS1 column, particle size 5 μm, was used. Gradient elution was carried out at a flow rate 0.8 ml/min keeping the column at 30 °C constant temperature. The starting eluent contained 86 % phosphate buffer (pH 2) and 14 % acetonitrile. After 48 minutes the proportion of acetonitrile reached 40 %. During the next 12 minutes the proportion of acetonitrile reached 70 % and then decreased to 11 % in three minutes. Between successive runs the column was stabilized by pumping this eluent through it for 15 minutes. The UV-spectra were measured from 200 to 420 nm. PDA-data was collected at the rate of 1 spectrum/second with the resolution of 2.4 nm. The amounts injected were 10 µl for all samples.

Model compounds

The UV-spectra of compounds obtained as oxidation products were compared to a library containing the spectra of more than two hundred phenolic model compounds. Many non-chlorinated model compounds were commercially available (Merck, Fluka and Aldrich). Chlorinated hydroxybenzaldehydes and benzoic acids used in making the libraries and calibration curves were synthesized as described before[47-50] or using methods which will be reported later. Synthesis routes for some important model compounds found among the oxidation products are given below.

6-chloroacetovanillone was synthesized applying the Fries rearrangement[51] of 5-chloroguaiacol acetate. 30 grams of guaiacol, 30 ml of acetanhydride and two drops of concentrated sulphuric acid were refluxed for 30 minutes. 50 ml of dichloromethane was added and the mixture was washed with 100 ml of water. Dichloromethane was removed and the guaiacol acetate was collected at 120-130 °C by distillation under reduced pressure. 20 grams of guaiacol acetate was dissolved in 50 ml of chloroform and 8 ml of sulphuryl chloride was added. The resulting solution was mixed at room temperature for 24 hours after which additional 8 ml of sulphuryl chloride was added. Using GC the solution was found to contain 50 % of 5-chloroguaiacol acetate and a trace amount of 4-chloroguaiacol acetate. After washing with water the mixture was distilled under reduced pressure and the crude 5-chloroguaiacol acetate (purity ca. 70 %) was collected at 130-135 °C. 2.0 g of 5-chloroguaiacol acetate and 3.3 g of powdered aluminium chloride were mixed and heated for one hour on a boiling water bath. The use of temperatures above 100 °C cause 6-chloro-3,4-dihydroxyacetophenone to form. After cooling the solid mixture was dissolved in 2 M HCl and diethyl ether. After separation in a separatory funnel the ether fraction was dried and evaporated to dryness. The product was purified by column chromatography on silica gel (Kieselgel 60, 70-230 mesh, Merck). A 95/5 (v/v) mixture of dichloromethane and ethanol was used as eluent. The yield was 0.19 g and melting point 104-106 °C, lit. 109-110 °C.[52]

Dehydrodivanillin and **dehydrodiacetovanillone** were synthesized from corresponding monomers modifying the method of Drumond *et. al.*[53] As follows, 12.0 g of vanillin was dissolved in 4 l of water and 80 ml of acetone. A mixture of g 10.0 g of $K_2S_2O_8$ and 0.8 g of $FeSO_4$ was added and the solution was stirred at ambient temperature for one week. The precipitated crude product was separated by filtration, recrystallized from pyridine and washed with petroleum ether to give 2.7 g of pure compound degrading at 280-290 °C. To prepare dehydrodiacetovanillone 3.0 g of acetovanillone was dissolved in 500 ml of water and 15 ml of acetone. After adding a mixture of 3.0 g $K_2S_2O_8$ and 0.2 g of $FeSO_4$ the solution was mixed for one week. The crude product was recrystallized from acetic acid and 1.1 g of pure dehydrodiacetovanillone was obtained, mp. 308-310 °C.

5-formylvanillin was synthesized by the formylation of guaiacol.[54] A mixture of 20.0 g of guaiacol, 20.0 g of paraformaldehyde and 20.0 g of hexamethylenetetramine was heated on an oil bath to 130 °C. Acetic acid (40 g) was added dropwise into the brown oil formed and the mixture was refluxed for 3 hours. 20.0 g of concentrated sulphuric acid was added to the mixture and the refluxing was continued for one hour. The cooled mixture was diluted with 100 ml of water and extracted several times with diethyl ether. After the evaporation of ether the crude product was recrystallized from toluene to give 2.3 g of pure product. Melting point of the product was 122-124 °C, lit. 125-126 °C.[55]

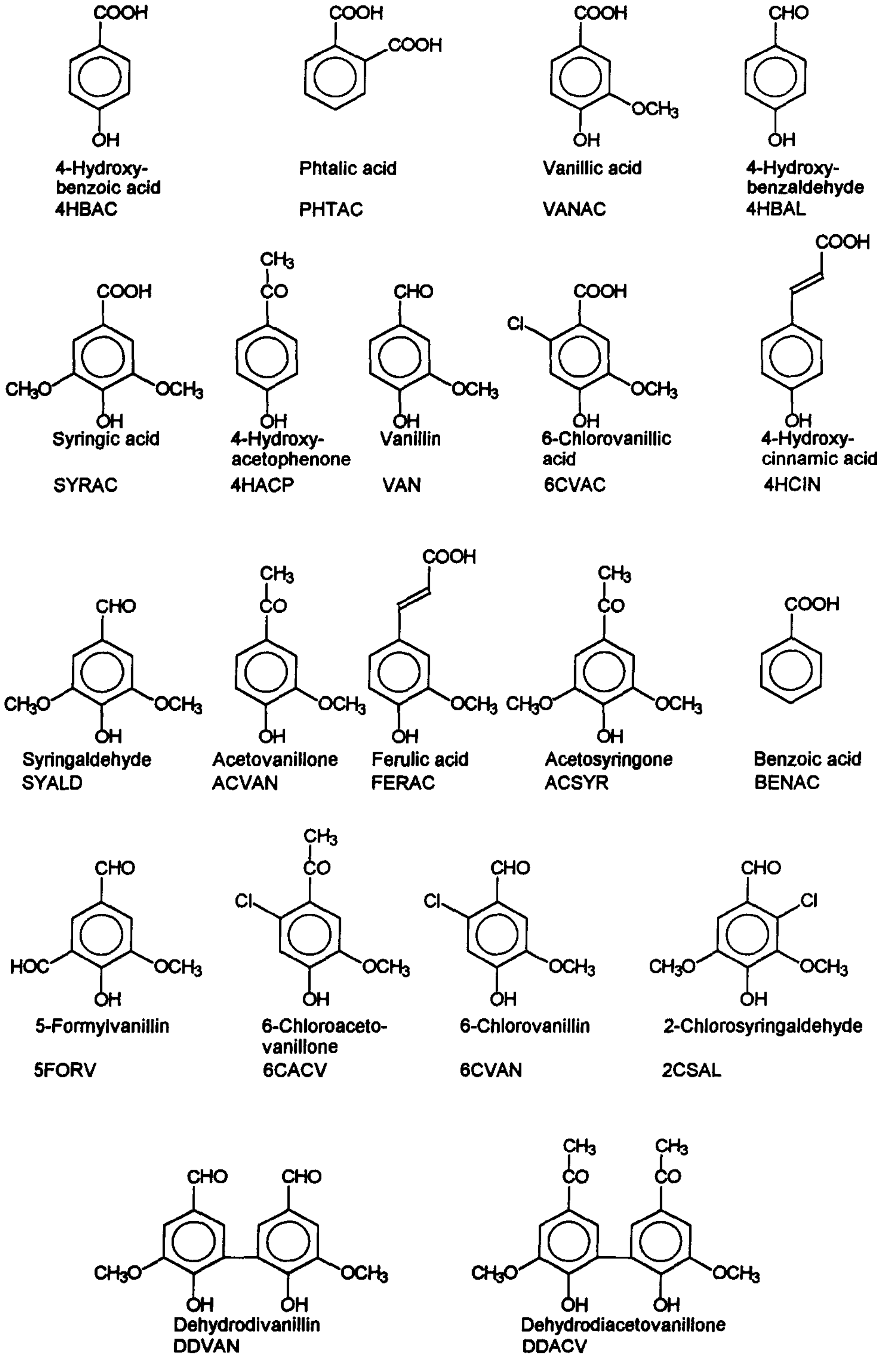
RESULTS AND DISCUSSION

The amounts of water samples ultrafilered and of high molecular mass material obtained by ultrafiltration are given in Table 1. As expected, the sample EFF contains much more high molecular mass material than the other samples studied do. Also the sample AIT contains more of these compounds than the waters downstream of the pulp mill. This can be explained by the brown coloured AIT sample containing high amounts of natural humic substances, but the volume of AIT water in the receiving water system is small compared to that of the clear water streaming from lake Keitele (see Figure 1). The relative amounts of identified oxidation products are collected in Table 2. Structures of the compounds are given in Figure 2. The identifications were based on the UV-spectra of model compounds and verified by the standard addition method. Examples of chromatograms are given in Figure 3.

Sample name	Sample amount, ml	M_n > 10 000, mg	M_n 1 000 - 10 000, mg
AIT	1000	22	20
EFF	200	72	64
KAP	1000	13	16
KUU	1000	13	17
HAA	1000	6	11
LEP	1000	7	10

Table 1. Yields obtained by ultrafiltration of the surface water samples and the pulp mill effluent

Because the CuO oxidation breaks down only part of all the high molecular mass compounds to identifiable monomers or dimers, it is not sensible to calculate the amounts of aromatic structures based on quantitations of compounds identified. The quantitations gave 2.5 to 1.0 % identifiable oxidation products from the mass of ultrafiltrated fractions oxidized. In sediments only 0.2 to 0.08 % of the original sample was transformed to identifiable oxidation products. On the other hand, the comparison of amounts, or more simply the ratios of peak heights in different samples, gives valuable information about the differences between the samples studied. Peak heights were used in the calculation of figures in Table 2 to prevent the interferring effects of impurities in the beginning or at the end of the peaks. Vanillin was chosen as the reference compound because it results in the largest peaks in most samples. On the other hand, the amount of vanillin differs from sample to sample causing difficulties in the interpretation of results. It was also observed that the amounts of identifiable compounds were higher in EFF than in any other sample. This indicates that the ratio of lignin based aromatic macromolecules to all macromolecules is higher in effluent than in the other water samples studied.



4-Hydroxybenzoic acid
4HBAC

Phtalic acid
PHTAC

Vanillic acid
VANAC

4-Hydroxybenzaldehyde
4HBAL

Syringic acid
SYRAC

4-Hydroxyacetophenone
4HACP

Vanillin
VAN

6-Chlorovanillic acid
6CVAC

4-Hydroxycinnamic acid
4HCIN

Syringaldehyde
SYALD

Acetovanillone
ACVAN

Ferulic acid
FERAC

Acetosyringone
ACSYR

Benzoic acid
BENAC

5-Formylvanillin
5FORV

6-Chloroacetovanillone
6CACV

6-Chlorovanillin
6CVAN

2-Chlorosyringaldehyde
2CSAL

Dehydrodivanillin
DDVAN

Dehydrodiacetovanillone
DDACV

Figure 2. Structures of the compounds identified from oxidation products

COMP.	$R_{t,ave}$/min	AIT	EFF	KAP	KUU	LEP	HAA	SEK	SEV	SEL
4HBAC	14.5	-	-	-	D	-	-	0.11	0.01	0.04
PHTAC	14.6	D	D	0.16	D	D	0.57	-	-	-
VANAC	17.1	0.33	0.13	0.22	0.25	0.20	0.17	0.31	0.10	0.27
4HBAL	18.3	0.51	0.08	0.40	0.36	0.35	D	1.01	0.16	0.42
SYRAC	18.8	0.12	0.03	0.05	0.05	0.05	D	0.29	0.03	0.18
4HACP	21.4	0.58	0.01	0.40	0.36	0.34	0.35	0.33	0.03	0.14
VAN	22.1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6CVAC	23.6	-	0.01	-	-	-	-	-	-	-
4HCIN	24.5	-	-	-	-	-	-	1.89	0.10	0.59
SYALD	24.7	0.07	0.07	0.10	0.06	D	0.06	0.11	0.04	0.08
ACVAN	25.3	0.37	0.19	0.33	0.31	0.29	0.31	0.40	0.16	0.31
ACSYR	27.2	0.17	0.05	0.15	0.12	0.11	0.14	0.19	0.04	0.10
FERAC	27.5	-	-	-	-	-	-	0.17	0.04	0.08
BENAC	28.3	D	D	0.02	0.01	-	D	-	-	-
5FORV	29.0	-	0.01	-	-	-	-	-	0.02	-
6CACV	38.9	-	0.01	-	-	-	-	-	-	-
6CVAN	40.0	-	0.16	0.05	0.07	0.06	0.04	-	0.02	0.04
2CSAL	40.5	-	0.04	0.03	0.01	0.01	0.01	-	-	-
DDVAN	42.2	0.10	0.06	0.10	0.11	0.09	0.09	D	0.08	D
DDACV	43.5	0.03	0.04	0.04	0.03	0.03	0.03	0.07	0.04	0.04
A	12.7	0.18	-	0.09	0.08	0.28	0.04	0.74	0.04	0.29
B	20.2	0.09	0.03	0.08	0.08	0.10	0.08	-	-	-
C	26.2	0.10	0.03	0.08	0.08	0.06	0.10	-	-	-

D: The compound was identified as an impurity of another compound and ratio is not calculated

Table 2. The relative amounts of compounds identified from the oxidation products

The comparison of AIT to EFF shows some characteristic differences. First, the appearance of chlorinated compounds (6-chlorovanillic acid, 6-chloroacetovanillone, 6-chlorovanillin and 2-chlorosyringaldehyde) is a clear indication of chlorolignins, in accordance with earlier studies.[11-14] Secondly, there is a very small but observable amount of 4-hydroxyacetophenone in EFF whereas in AIT it is one of the most abundant compounds. On the other hand, the peak of vanillin is clearly more prominent in the oxidation products of downstream samples than in AIT. Thus, the 4-hydroxyacetophenone to vanillin ratio can be used to follow the transport of macromolecular compounds produced in the pulp mill. AIT and HAA are very similar, indicating that most of the macromolecular compounds of the pulp mill effluent have either degraded, diluted or sedimentated during the 40 km stretch downstream of the mill. However, 6-chlorovanillin and 2-chlorosyringaldehyde could still be detected in HAA (see Figure 3).

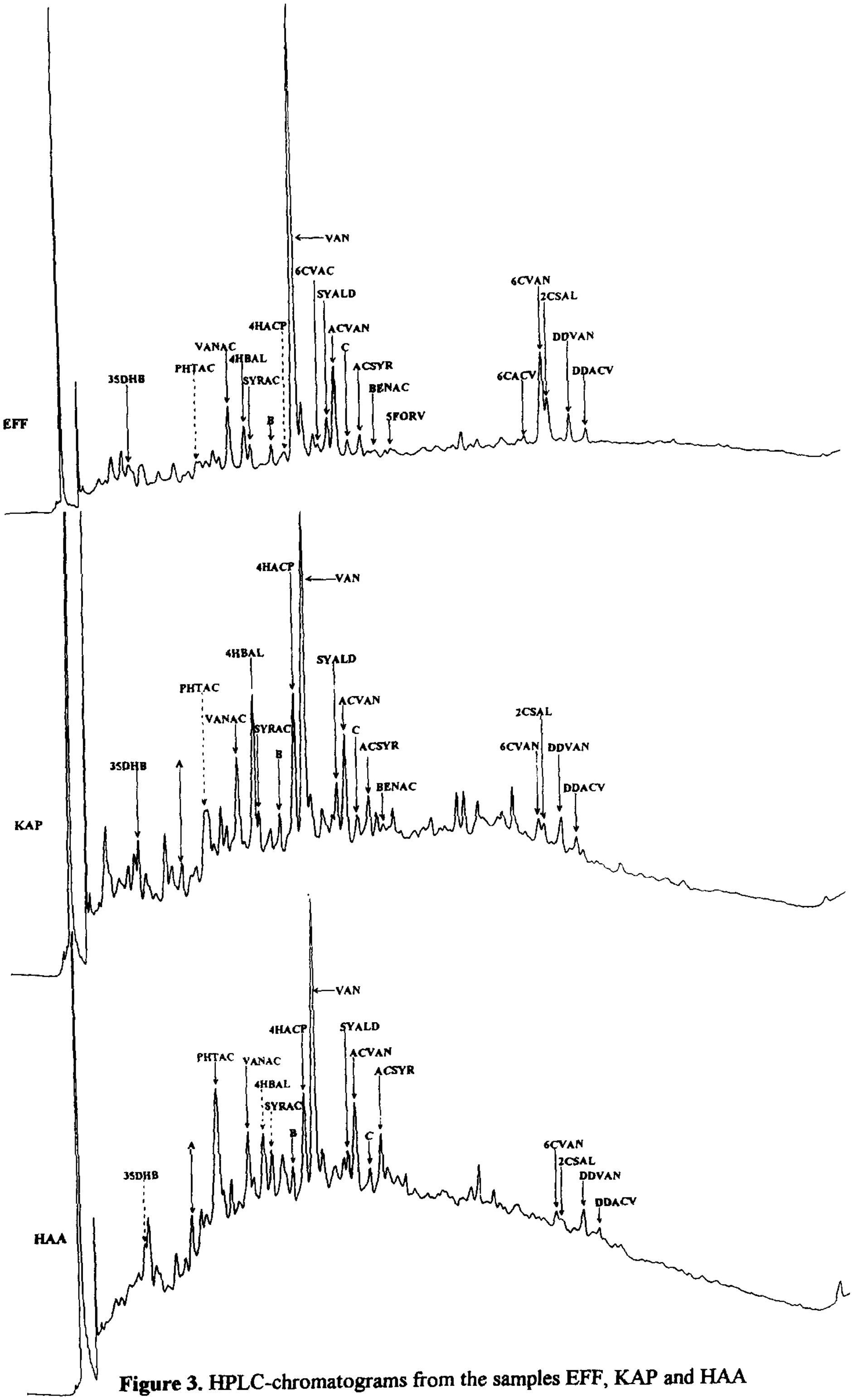


Figure 3. HPLC-chromatograms from the samples EFF, KAP and HAA

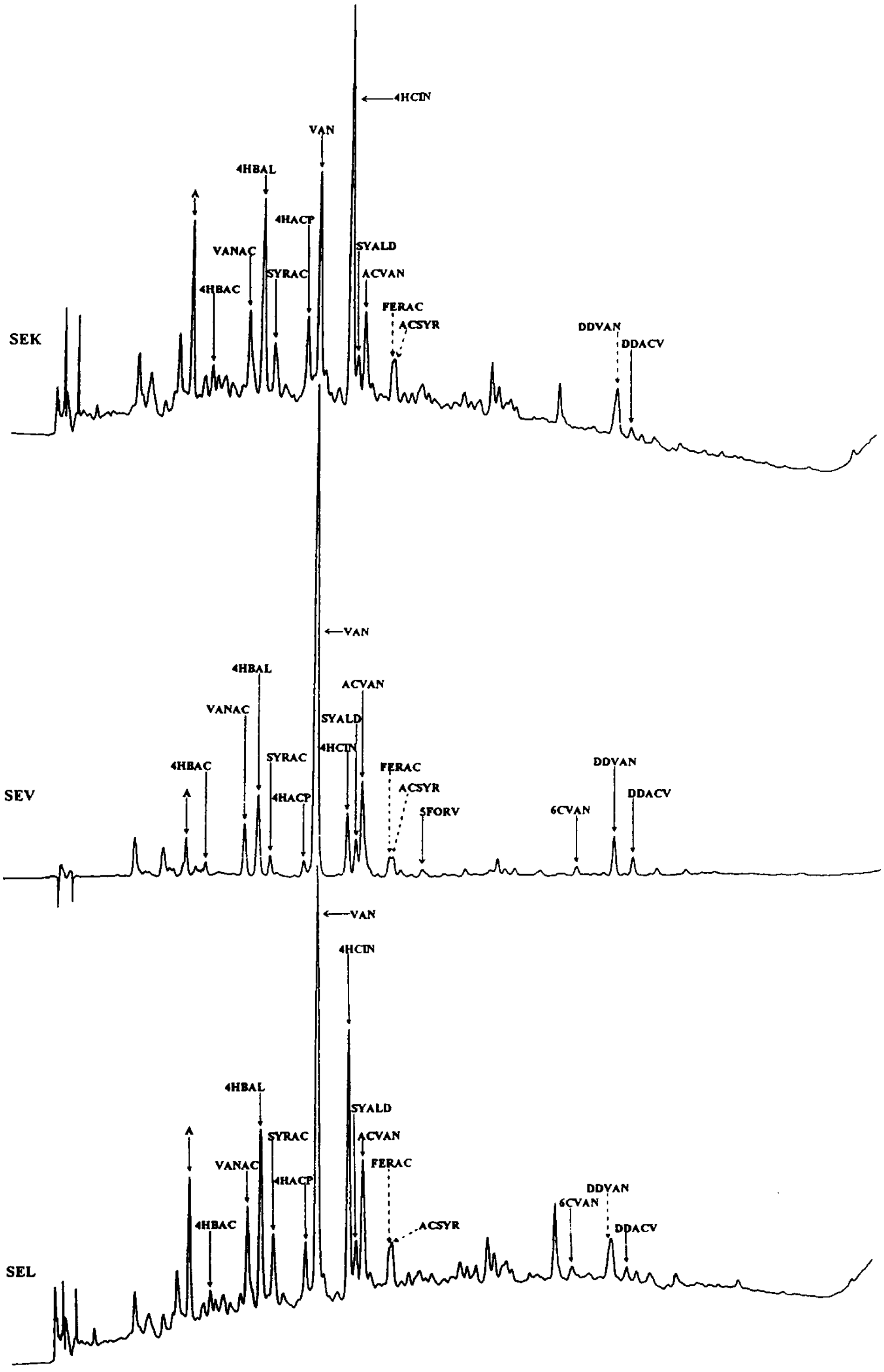


Figure 4. HPLC-chromatograms from the samples SEK, SEV and SEL

Figure 4 shows the HPLC fingerprints of sediment samples oxidized. Comparison of SEK, SEV and SEL shows that the differences are about the same as in the oxidation products of samples separated from water. Only one chlorinated compound (6-chlorovanillin) could be identified from SEV and SEL. The 4-hydroxyacetophenone to vanillin ratio is very small in SEV and has not increased to the background level in SEL.

The most important differences between the oxidation products of samples from water to those of sediments are the appearance of 4-hydroxycinnamic acid and ferulic acid and the smaller amounts of chlorinated compounds in the latter. These findings might indicate the presence of structures with unsaturated side chain in the high molecular mass material of sediment samples and that the chlorine atoms are either mostly bound to the more oxidized and more water soluble macromolecules or that they gradually degrade in the sediments. Recently, we have observed high contents of ferulic, p-coumaric and cinnamic acids among the oxidation products of bitumen fractions separated from composted materials.[56] The presence of substituted cinnamic acids is also interesting because, according to our studies with model compounds, they should readily oxidize in cupric oxide oxidation.

The oxidation products still contain many unidentified compounds characteristic to some samples analyzed. In Table 2, three of these compounds, marked A, B and C, are shown as examples. Compound A is characteristic to the oxidation products of sediments being one of the highest peaks in SEK and SEL. Compounds B and C could not be identified from SEK, SEV or SEL (Figure 4), but they are formed in the oxidation of all other samples (Figure 3). The UV-spectra of these compounds are shown in Figure 5.

CONCLUSIONS

The results obtained are logical indicating that the present method, together with other methods, is applicable to the characterization and to studying the transport of high molecular mass compounds in the receiving water systems of pulp mills. Unfortunately, dredging on the watercourse shortly before sampling, in the connection with building a canal between the lakes Päijänne and Keitele, must have caused some disturbance because the present results differ slightly from results obtained in preliminary tests one year earlier. Also, the most interesting sediment samples closer to the pulp mill could not be collected due to the fast flow of water.

As found in our earlier investigations, the method we used is reproducible. However, it can hardly be recommended as a routine procedure owing to the long time consumed in ultrafiltration and freeze drying. If the concentration of macromolecular compounds in water samples were high enough, they could be oxidized without ultrafiltration and freeze drying, but this procedure can only be applied to process waters and effluents. In future, the method will be developed further and other procedures will also be tested for the analysis of high molecular mass compounds in receiving waters and sediments. Additional model compounds will be synthesized and purchased in order to identify new compounds among the degradation products.

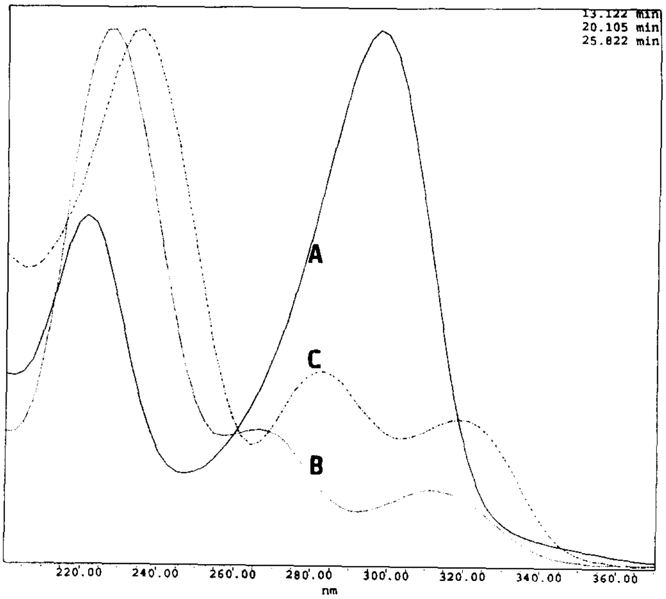


Figure 5. UV-spectra of unknown compounds A, B and C

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