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Combined high-performance aqueous size-exclusion chromatographic and pyrolysis-gas chromatographic-mass spectrometric study of lignosulphonates in pulp mill effluents

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ABSTRACT

An aqueous high-performance size-exclusion chromatographic (HPASEC) method for lignosulphonates was developed and optimized using a TSK G3000SW column. Curie-point pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) was used to characterize the lignosulphonates on the molecular level. Combined HPASEC and Py-GC-MS results showed that a sodium lignosulphonate standard, prepared under mild conditions, was a relatively polydisperse polymer that still contained features of a natural lignin polymer. Lignosulphonates discharged by pulp mills are more monodisperse macromoles of a relatively low molecular mass and are structurally modified to a greater extent than the sodium lignosulphonate standard. A relationship was established between the molecular mass distributions of the various lignosulphonate macromolecules and the fraction of preserved phenylpropane structural units.

INTRODUCTION

Lignosulphonates are formed during sulphite pulping processes of wood chips by substitution of α- or y-hydroxy groups on the side-chains of the 4-propanol-2-methoxyphenol and 4-propanol-2,6-dimethoxyphenol structural units of lignin [1]. The aim of sulphite pulping is to extract lignins from wood by sulphonation, yielding a brighter wood pulp [1]. Although the toxicity of lignosulphonates is probably low, this class of compounds may significantly contribute to the sulphur content of river and drinking waters [2]. In modem sulphite pulp mills, the emission of lignosulphonates has been strongly reduced by recovery and commercial use or incineration of these compounds [3]. However, significant residual amounts of lignosulphonates are still discharged by modern sulphite pulp mills and very large amounts may still be discharged by older sulphite pulp mills [3,4].

The molecular mass distribution, as measured by size-exclusion chromatography (SEC), is an important parameter for characterizing this class of lignin-derived compounds. Lignosulphonates

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are largely composed of macromolecules having molecular masses of more than 1000 g mol^{-1}. Molecular mass distributions are used to characterize these water-soluble polymers and monitor structural changes during physical, chemical or biological degradation processes.

Aqueous SEC of lignosulphonates has been performed with organic polysaccharide-based Sephadex gels [5]. Over the past decade, silica-based high-performance aqueous SEC (HPASEC) gels have been developed which give higher plate numbers and increased analysis speed compared with polymeric gels [6–8]. A major advantage of HPASEC is that derivatization of water-soluble polymers is not necessary. This leaves macromolecular structures unchanged and allows the direct analysis of aqueous samples. An SEC study of aquatic humic substances using a TSK G3000SW column has been reported by Vartiainen et *al.* [9]. Up to now, no studies have been reported on aqueous SEC of lignosulphonates using this column.

The structural analysis of dissolved macromolecular lignosulphonates and chlorolignosulphonates in natural and waste waters has been reviewed recently [4]. Although chemical degradation techniques have frequently been used to identify substructures of lignosulphonates [10,11], these methods are time consuming and various chemical modifications of the phenylpropane structural units occur owing to the severe chemical conditions used in these methods.

Curie-point pyrolysis-gas chromatography–mass spectrometry (Py-GC-MS) is a rapid microanalytical method for the structural analysis of lignin polymers on a molecular level [12,13]. The technique requires minimum sample preparation and preserves side-chain information of the phenylpropane structural units. Py-GC-MS has been applied to pulp mill effluents [4,14], chlorolignins in xylan [15] and plastic contaminants in pulp [16].

The objectives of this study were to develop an optimized HPASEC method to determine molecular mass distributions of lignosulphonates using the TSK G3000SW column. Curie-point Py-CG-MS was used to obtain detailed structural information on a molecular level. The relationship between the molecular mass of the various lignosulphonate macromolecules and the preservation of phenylpropane structural units was investigated.

EXPERIMENTAL

Chromatographic conditions

The SEC system consisted of an Applied Biosystems (Ramsey, NJ, USA) Model 400 isocratic pump, a Rheodyne (Berkeley, CA, USA) Model 7125 injection valve with a 20-μl sample loop and an Applied Biosystems Model 783 UV detector (280 nm), which was connected in series with a Spectra-Physics (San Jose, CA, USA) Model SP 6040 XR refractive index (RI) detector. The system was operated at ambient temperature. The use of a Waters TCM column oven (50°C) did not improve the chromatographic performance of the system.

A TSK $G3000SE_{XL}$ precolumn (750 mm × 7.5 mm I.D.) was used in combination with a TSK $G3000SW_{XL}$ analytical column (300 mm × 7.5 mm I.D.) (Toyo Soda Manufacturing, Tokyo, Japan). Its theoretical plate number, determined with ethylene glycol (Merck, Darmstadt, Germany), was 41400. The void volume (V_0), determined with a pullulan standard (molecular mass 853 000 g/mol), was 5.74 ml. The permeation volume (V_t), determined with ethylene glycol, was 12.71 ml. Molecular mass determinations were obtained using a 0.2 *M* sodium acetate (Merck) mobile phase at a flow-rate of 1 ml/min. The mobile phase was adjusted to pH 7 with nitric acid (J.T. Baker, Deventer, Netherlands), filtered (0.45-μm filter, Millipore) and purged with helium.

Data were processed using a Nelson 760 series interface and Nelson Analytical software (Version 5.1) and laboratory-made SEC software (FOM-Amolf, Amsterdam, Netherlands).

Curie-point pyrolysis mass spectrometry

Curie-point pyrolysis was performed with a FOM 4-LX pyrolysis unit [12]. Sample solutions of 20 μg (5 μl of aqueous solution of 4 mgfml) were applied to a ferromagnetic wire and dried under reduced pressure. The wire was inserted in a glass liner, flushed with argon to remove air

and subsequently placed into the pyrolysis unit. The ferromagnetic wire was inductively heated within 0.1 s to its Curie-point temperature (610°C), at which it was held for 4 s. Pyrolysis fragments were flushed to a 25 m × 0.32 mm I.D. CP-SIL-5 CB fused-silica capillary column (film thickness 0.41 μm) using helium as a carrier gas. Py-GC-MS was performed using a Packard 438 S chromatograph coupled to a Jeol DX-303 double-focusing (EB) mass spectrometer. The GC oven was kept at 30°C during pyrolysis and was subsequently programmed to 300°C at 4°C/min. The interface was kept at 200°C and the ion source at 180°C. Compounds were ionized at 70 eV under electron impact conditions and mass analysed over the range m/z 35-500. Data were processed using the Kratos (Manchester, UK) analytical MACH3 software package.

Materials

Sodium polystyrenesulphonate (NaPSS) of molecular mass 1200000, 400 000, 46000, 18000, 8000, 4600 and 1800 g/mol and pullulan standards of molecular mass 853 000, 380000, 100000, 48 000, 23 700 and 5800 g/mol were obtained from Polymer Laboratories (Church Stretton, UK). Maltoheptaose of molecular mass 1152 (Boehringer, Mannheim, Germany) and maltotriose of molecular mass 504 (Serva, Heidelberg, Germany) were used as low-molecular mass polysaccharide standards.

Sodium lignosulphonate was purchased from Roth (Karlsruhe , Germany). Deionized water obtained with a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout.

Samples from German sulphite pulp mill effluents were collected in January 1989 from PWA Aschaffenburg, Holtzmann (Karlsruhe) and PWA Mannheim. The samples were stored in high-density polyethylene containers (Nalgene, Rochester, NY, USA) with a low phthalate content and refrigerated at -20°C.

RESULTS AND DISCUSSION

Owing to the heterogeneous composition of lignosulphonates, no universal monodisperse calibration standards are available. Molecular mass calibration with polymer standards which are structurally different from the polymers under investigation requires the assumption that the chromatographic separation is governed by the hydrodynamic volume ($[\eta]M$) only. The structural resemblance between the sample solutes and the calibration standards essentially determines the accuracy of the calibration results. Although ultrafiltration fractions of lignosulphonates probably give more representative calibration standards [17], this method is time consuming and apparently less reproducible, owing to differences in the selected samples and ultrafiltration systems. Therefore, well defined NaPSS standards which show an acceptable structural resemblance to lignosulphonates were preferred in this study.

Rigid, porous silica-based hydrophilic gels such as TSK type SW gels are known to contain residual silanol groups as a result of incomplete derivatization [6,7]. These anionic groups ($pK_a \approx 7$ [18]) give rise to secondary separation effects of polar and ionized solute molecules and lead to deviations in calibration results [7,8,19].

Ion-exclusion effects caused by repulsion of negatively charged solutes from the pores of the TSK SW gel by dissociated silanol groups were investigated by comparing the calibration graphs of NaPSS and pullulan using different eluent ionic strengths. Fig. 1 shows the linear part of the calibration graphs (3500-100000 g/mol) at various ionic strengths of sodium acetate. Apparently, the pullulan calibration graph is independent of the ionic strength, *i.e.*, this linear uncharged polymer is not subject to ion exclusion. However, the NaPSS calibration graph is strongly dependent on the eluent ionic strength. This demonstrates strong electrostatic interactions between sulphonate functional groups and the packing surface. As a result, earlier elution of NaPSS relative to a non-ionized pullulan standard of the same molecular mass is observed.

A theoretical ion-exclusion graph can be derived from the Debye–Hückel theory [19]:

$$V_e = kI^{-1/2} \quad (1)$$

where V_e is the ion-exclusion volume, k is a fitting factor and Z is the ionic strength of the mobile phase. By choosing an appropriate value for k (*i.e.* 2.34 for molecular mass 1800), the

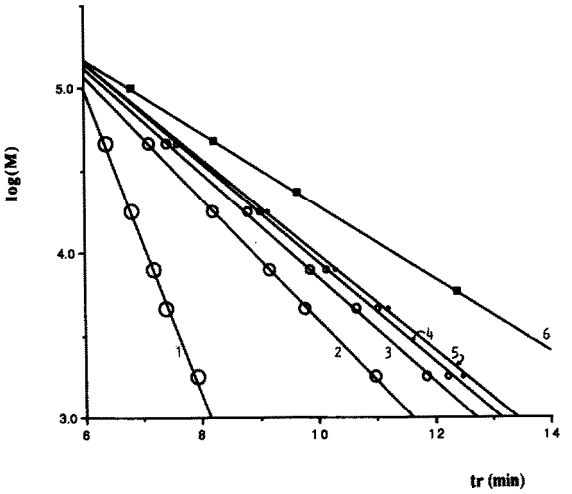

Fig. 1. Optimization of the mobile phase ionic strength using pullulan (6) and sodium polystyrenesulphonate standards. Mobile phase: 0.01 *M* sodium acetate (1); 0.05 *M* sodium acetate (2); 0.1 *M* sodium acetate (3); 0.15 *M* sodium acetate (4); 0.2 *M* sodium acetate (5). pH = 7.

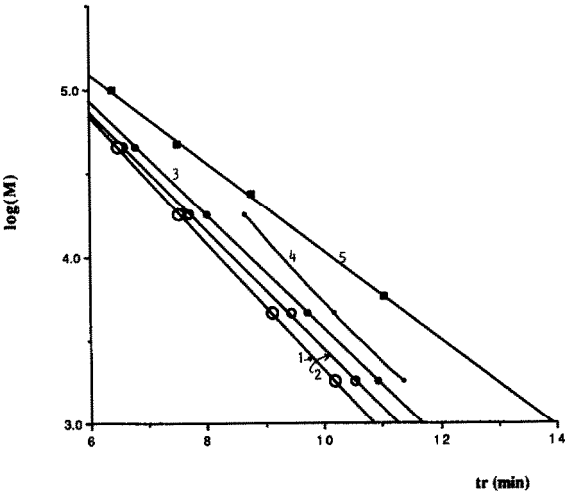

Fig. 2. Optimization of the mobile phase pH using pullulan (5) and sodium polystyrenesulphonate standards. Mobile phase: 0.05 *M* sodium acetate, pH 7 (I), pH 6 (2), pH 5 (3) and pH 3.8 (4).

Debye–Hückel graph can be fitted very well on to the experimental data. As significant deviations from this theoretical graph usually indicate adsorption effects, it can be concluded that ion exclusion is the only secondary separation effect of NaPSS using the present type of column at pH 7. Fig. 1 shows that this effect is minimized at high ionic strengths.

The effect of cations on the separation was investigated with eluents containing 0.01 *M* sodium acetate, potassium acetate or ammonium acetate. The type of cations in the eluent did not influence the calibration graph of NaPSS or the chromatography of the lignosulphonate samples. This indicates that the hydrodynamic volume of the cations plays an insignificant role in the shielding of the ionized silanol and sulphonate groups.

The effect of eluent pH is shown in Fig. 2. The mobile phase pH was optimized using 0.05 *M* sodium acetate buffers with pH values of 3.8, 5, 6 and 7. Interestingly, the calibration graph of NaPSS shifts towards the pullulan calibration graph when the eluent pH is lowered. This can be explained by reduced ion exclusion, due to protoriation of silanol groups. Barth [7] reported that below pH 4 the ionization of silanol groups is suppressed. When an eluent pH of 3.8 was used, irreversible adsorption of high-molecular mass NaPSS standards on the stationary phase was observed in our study. This indicates strong hydrophobic interactions between NaPSS and the TSK G3000SW gel, probably as a result of hydrogen bonding of free phenolic hydroxyl groups with protonated silanol groups. Protonation of the sulphonate groups is unlikely at pH 3.8 because lignosulphonates are strongly acidic macromolecules (ply, ≈ 0.5 [20]). The decrease in ion-exclusion effects on decreasing the eluent pH from 7 to 5 is comparable to the increase in the ionic strength from 0.05 to 0.1 M. Changing the pH may also lead to deviations in measured molecular mass distributions as a result of the formation of association complexes. This effect is minimized at pH 7-9 and at low dissolved organic carbon concentrations (<100 mg/l) [21,22].

In general, improved chromatographic resolution is observed when an eluent of low ionic strength is used. The best resolution was obtained using 0.01 *M* sodium acetate. In Fig. 3, characteristic HPASEC traces are shown which allow "fingerprinting" of the lignosulphonate samples. Decreasing the electrolyte concentration will also lead to an increase in intramolecu-

lar expansion of polyanionic lignosulphonates [7]. As a result of these inter- and intramolecular electrostatic effects at low electrolyte concentrations, a dramatic decrease in retention volume of most compounds is observed (Fig. 3) compared with the chromatograms obtained at 0.2 M sodium acetate (Fig. 4), and no accurate molecular mass determinations are obtained. Gupta and McCarthy [23] showed that the effective hydrodynamic radius of lignosulphonates was approximately doubled when the electrolyte concentration was changed from 1.0 M NaCl to zero in water.

It appears from the present data that by using an eluent ionic strength of 0.2 M, sodium acetate ionic effects are strongly reduced. To avoid the possibility of increasing adsorption and association at low eluent pH (<4), the increase in ionic strength was preferred to suppress secondary separation effects. Thus, an eluent of 0.2 M sodium acetate and pH 7 was used for molecular mass determinations.

The reproducibility of the system was investigated with respect to retention time, peak height and peak area by seven injections of an NaPSS standard (molecular mass 1800). The relative standard deviations were 0.2, 2 and 5%, respectively.

HPASEC traces of the sodium lignosulphonate and pulp mill effluent samples recorded by UV absorption are shown in Fig. 4. The corresponding number-average molecular masses (M_n) and mass-average molecular masses (M_w) are listed in Table I. The sodium lignosulphonate standard shows a relatively high M_w value compared with the lignosulphonates discharged by pump mill effluents, whereas the M_n values obtained are comparable. A useful procedure is to define the ratio M_w/M_n as a measure of the polydispersity of the sample. The corresponding

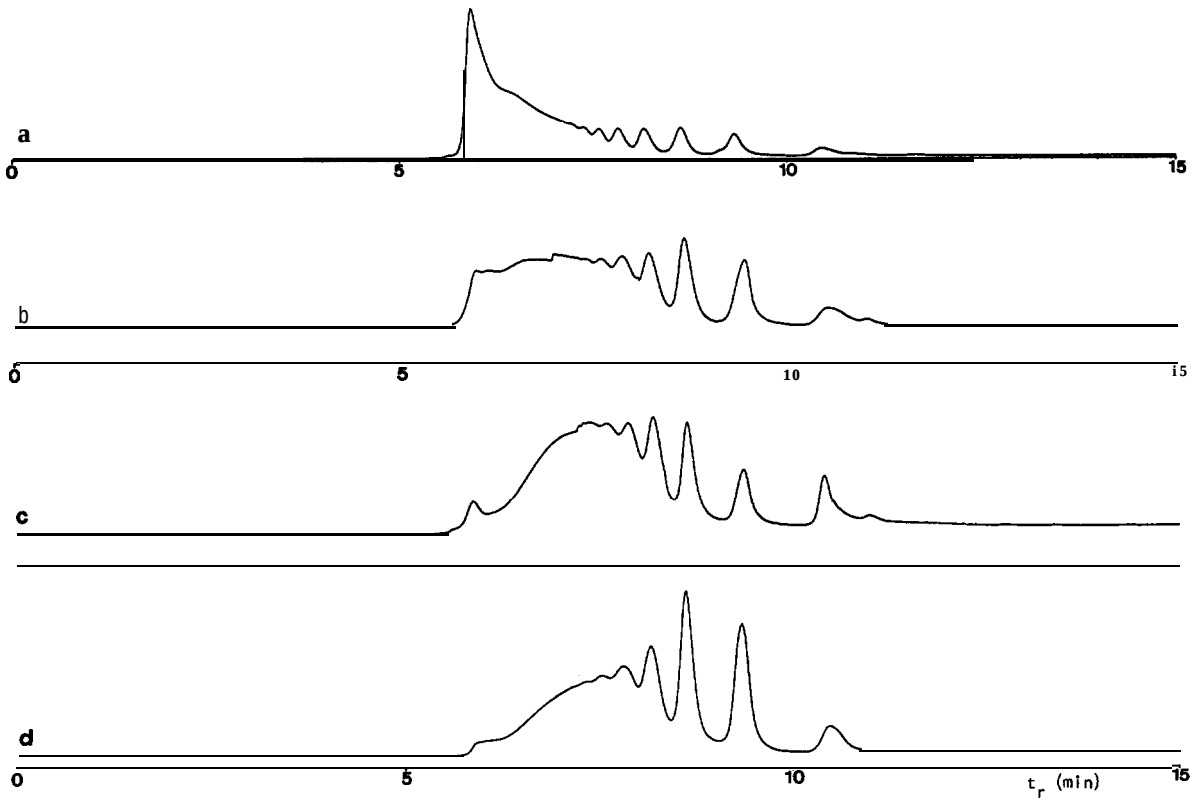

Fig. 3. Molecular mass distributions of (a) sodium Egnosulphonate, (b) effluent from PWA Mannheim, (c) effluent from Holtzmann and (d) effluent from PWA Aschaffenburg. Eluent: 0.01 M sodium acetate (pH 7).

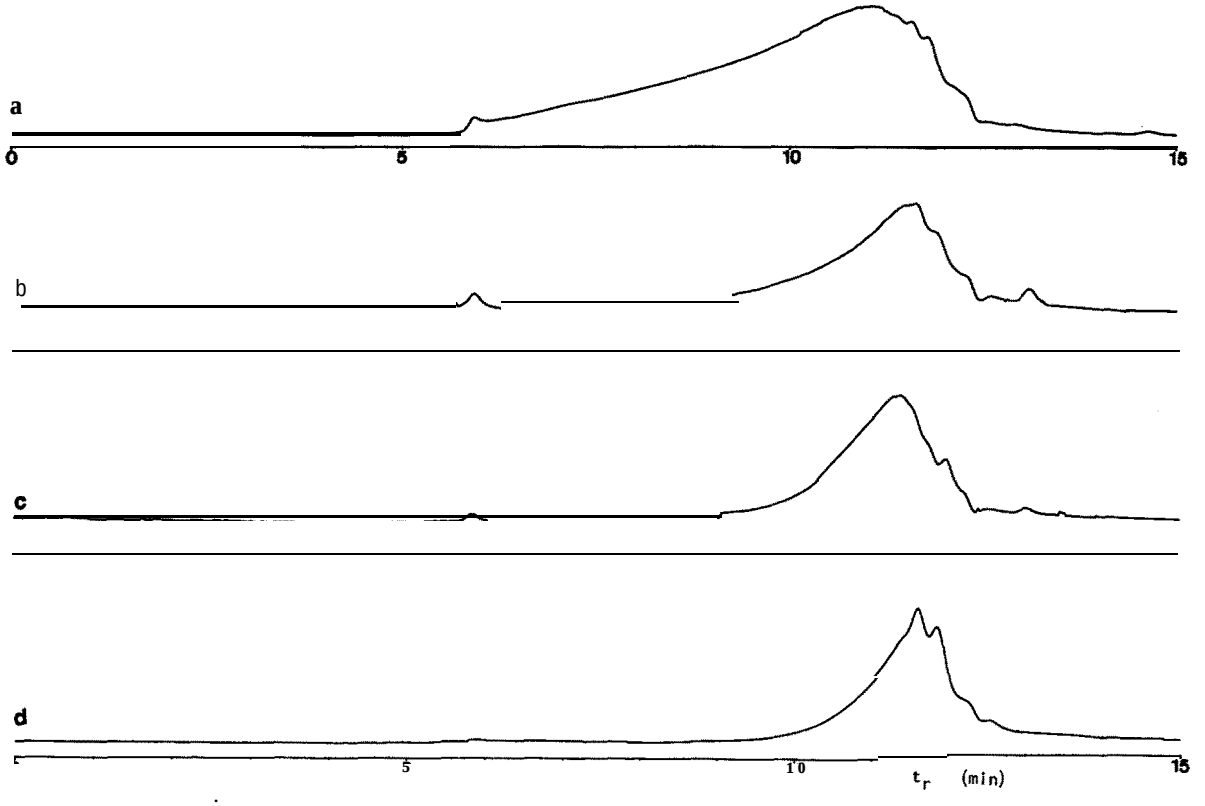

Fig. 4. Molecular mass distributions of (a) sodium lignosulphonate, (b) effluent from PWA Mannheim, (c) effluent from Holtzmann and (d) effluent from PWA Aschaffenbnrg. Eluent: 0.2 *M* sodium acetate (pH 7).

polydispersity index values (*PD*) [24] are given in Table I.

Large *PD* values, indicating a wide polydispersity of molecular mass, are characteristic of non-linear polymers [25]. The *PD* value of 6.12 obtained for the sodium lignosulphonate standard indicates that a wide range of molecular sixes is obtained in the sulphonation process but that depolymerization is not taken to the stage at which the product consists of monomer and low-molecular-mass oligomers. The pulp mill effluent samples show much lower *PD* values than the

TABLE I

HPASEC AND Py-GC-MS RESULTS FOR SODIUM LIGNOSULPHONATE AND LIGNOSULPHONATES DISCHARGED BY PWA MANNHEIM, HOLTZMANN AND PWA ASCHAFFENBURG

Sample	M_w (g/mol)	M_n (g/mol)	Max. M_w (g/mol)	*PD*[a]	*DP*[b]	>5000[c] (%)	C_6C_3[d] (%)
Sodium lignosulphonate	16 700	2700	144300	6.12	7.5	57	49
PWA Mannheim	8400	3300	20 700	2.55	38	43	38
Holtzmamr Karlsruhe	4900	3ooo	9700	1.67	22	29	33
PWA Aschaffenburg	4100	2600	11300	1.61	18	27	32

[a] Polydispersity, M_w/M_n.
[b] Degree of polymerization (average structural unit masses 223 g/mol).
[c] Cumulative fraction >5000 g/mol.
[d] Cumulative fraction of phenylpropane structural units as determined by Py-GC-MS.

sodium lignosulphonate standard, indicating more monodisperse distributions of lignosulphonates formed during the sulphite pulping processes. Large differences in the maximum molecular mass (Table I) which could be observed in the HPASEC traces are also reflected in the polydispersity factors. The calibration results listed in Table I clearly show that the sodium lignosulphonate standard mainly consists of macromolecules of molecular mass above 5000 g/mol, whereas the pulp mill effluents appear to be composed of more depolymerized lignosulphonates. The approximate degree of polymerization (DP) was calculated (Table I) by taking into account that lignosulphonates usually contain one $-SO_3Na$ functional group for each two structural units [1], and the average molecular mass of one monomer unit is consequently about 223 g / mol for softwood-derived lignosulphonates.

Differences in aliphatic side-chain types are an important criterion for the degree of modification of the original phenylpropane (C_6C_3) structural units during sulphite pulping. Analytical flash pyrolysis was used to dissociate lignosulphonates thermally into cleavage products which reflect the structure of the original polymer [12]. The Py-GC-MS trace of sodium lignosulphonate is shown in Fig. 5. The distributions of the 2-methoxyphenol structural units obtained on pyrolysis are given in Table II. The major peaks observed in the chromatogram are assigned to 2-methoxyphenol **(1), 2-methoxy-4-vinylphenol (4), *trans*-2-methoxy-4-(prop-2-enyl)phenol (9), 2-methoxy-4-(propan-2-one)phenol** (13) and 2-methoxy-4-(propanol) phenol (16). Sulphonate groups which occupy the α- or y-position of the aliphatic side-chain of the lignin structural units are readily eliminated during pyrolysis. Hence a relatively high abundance of pyrolysis products with unsaturated aliphatic side-chains is observed.

The lignosulphonate pyrolysis products with preserved C_6C_3 structural units, listed in Table I, show a definite trend with respect to the molecular mass distributions. A total amount of 49% of intact phenylpropane structural units was obtained on Py-GC-MS analysis of sodium lignosulphonate. Lower fractions of preserved C_6C_3 structural units are obtained on pyrolysis of the lignosulphonates discharged by pulp mills (Table I). This indicates that a large proportion of the propane side-chains is dissociated during the pulping processes as a result of the cleavage of the interunit linkages in lignin. Small amounts (<1 %) of **2,6-dimethoxyphenol** derivatives are detected in the lignosulphonates discharged by PWA Mannheim and Holtzmann, which **indi-**

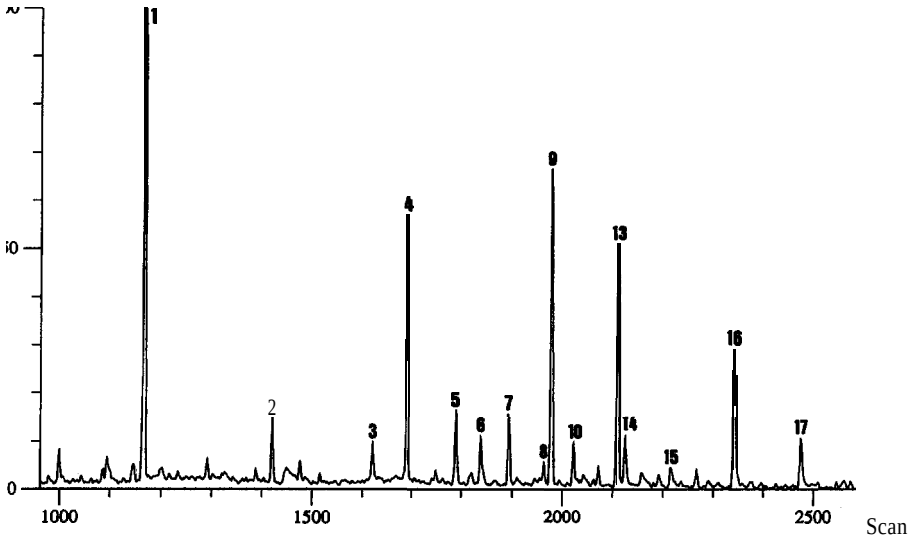

Fig. 5. Curie-point pyrolysis Py-GC-MS trace of sodium lignosulphonate. Peak numbers refer to Table II.

TABLE II

CURIE-POINT Py-GC-MS DATA FOR 2-METHOXYPHENOL PYROLYSIS PRODUCTS OF SODIUM LIGNOSULPHONATE (NaLS) AND LIGNOSULPHONATES DISCHARGED BY PWA MANNHEIM (A), HOLTZMANN KARLSRUHE (B) AND PWA ASCHAFFENBURG (C)

Peak No. [a]	Retention time (min)	Compound	Abundance (%)			
			NaLS	A	B	C
1	19.21	2-Methoxyphenol	23.6	19.7	18.0	17.4
2	23.38	2-Methoxy-4-methylphenol	3.2	8.9	12.4	12.2
3	27.02	2-Methoxy-4-ethylphenol	2.8	6.5	9.9	8.6
4	28.10	2-Methoxy-4-vinylphenol	12.7	11.8	20.4	17.5
5	29.50	2-Methoxy-4-(prop-1-enyl)phenol	3.9	4.5	4.9	3.0
6	30.41	2-Methoxy-4-formylphenol	2.8	12.1	4.1	10.2
7	31.36	2-Methoxy-4-(prop-2-enyl)phenol (*cis*)	3.6	2.1	2.4	3.4
8	32.40	2-Methoxy-4-(ethanal)phenol	1.1	–		
9	33.02	2-Methoxy-4-(prop-2-enyl)phenol *(tram)*	14.9	8.9	10.3	12.9
10	33.47	2-Methoxy-4-acetylphenol	2.2	2.9	2.1	2.6
11	33.54	2-Methoxyphenol-4-(C_3H_3) derivative	–	5.3	5.9	3.5
12	34.08	2-Methoxyphenol-4-(C_3H_3) derivative		4.0	4.3	4.2
13	35.15	2-Methoxy-4-(propan-2-one)phenol	13.1	5.9	5.2	3.3
14	35.26	2-Methoxy-4-(ethanol)phenol	2.6	0.4	–	
15	37.05	2-Methoxy-4-(propanal)phenol	1.6	1.8	–	1.2
16	39.05	2-Methoxy-4-(propanol)phenol	8.7	2.5	–	–
17	41.26	2-Methoxy-4-(prop-2-enal)phenol	3.2	2.7	–	–

[a] See Fig. 5.

cates that only softwood is used in the pulping process. Only the lignosulphonates discharged by PWA Aschaffenburg contain a mixture or copolymer of 2-methoxyphenol and 2,6-dimethoxyphenol lignin. A 2-methoxyphenol/2,6 dimethoxyphenol ratio of 0.7 was calculated from the Py-GC-MS data by summing all 2-methoxyphenol and 2,6-dimethoxyphenol pyrolysis products. The lignosulphonates present in the effluent of PWA Aschaffenburg contain 31% of 2-methoxyphenol derivatives with intact propane side-chains and 38% of 2,6-dimethoxyphenol derivatives with intact C_6C_3 structural units such as 2-methoxy-4-(prop-1-enyl)phenol, *trans*-2-methoxy-4-(prop-2-enyl)phenol and 2-methoxy-4-(propan-2-one)phenol. These results suggest that the 2-methoxyphenol structural units are more reactive towards sulphonation and hydrolysis reactions than 2,6-dimethoxyphenol structural units. A total amount of 32% of preserved phenylpropanoid structural units was calculated for the lignosulphonates present in the effluent of PWA Aschaffenburg.

Phenol and dihydroxybenzene derivatives are also detected in the pyrolysates of the lignosulphonates discharged by pulp mills such as phenol, 4-methylphenol, 4-ethylphenol, 4-vinylphenol, dihydroxybenzene and 4-methyldihydroxybenzene. These compounds are not detected in the mildly isolated sodium lignosulphonate standard used in this study. The relatively high abundance of these compounds in combination with the low abundance of preserved phenylpropane structural units clearly indicates that the original lignin polymer has been structurally changed as a result of demethylation, demethoxylation and condensation reactions which occur during the pulping processes. A detailed list of all the pyrolysis products observed on Py-GC-MS analysis of lignosulphonates in the pulp mill effluents used in this study has recently been published by Van Loon et al. [4].

Residual polysaccharides are detected in all effluent samples. Small amounts of chlorinated phenolic pyrolysis products are observed in the effluents of PWA Mannheim and Holtzmann such as 2-methoxy-6-chlorophenol, 2-methoxy-

4-methyl-6-chlorophenol, 2-methoxy-4-vinyl-6-chlorophenol, 2-methoxy-4-(prop-2-enyl)-6-chlorophenol, 2-methoxy-4-(propan-2-one)-6-chlorophenol and 2-methoxy-4-(chloropropyl)phenol. These chlorinated lignin structural units are formed during the bleaching sequences which are applied to the paper pulp in order to remove residual lignins.

CONCLUSIONS

Several secondary separation effects, which occur when using the TSK G3000SW column, were investigated and could be strongly reduced using a mobile phase of 0.2 *M* sodium acetate at pH 7. The molecular mass distributions show distinct differences between the various lignosulphonate samples and can be used to characterize structural modifications.

HPASEC traces obtained with 0.01 *M* sodium acetate as the eluent are characteristic and allow "fingerprinting" of sulphite pulp mill effluents. The relatively high chromatographic resolution makes this system potentially useful for fractionation and subsequent spectrometric analysis of lignosulphonates.

The combined HPASEC and Py-GC-MS data show that the sodium lignosulphonate standard, which is prepared under relatively mild conditions, is a relatively polydisperse polymer with a large proportion of preserved phenylpropane structural units. This water-soluble macromolecule still contains features of a natural lignin polymer. The lignosulphonates discharged by paper pulp mills are more monodisperse macromolecules of a lower molecular mass which are modified to a greater extent.

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REFERENCES

1 D.W. Glennie, in K.V. Sarkanen and C.H. Ludwig (Editors), *Lignins*, Wiley, New York, 1971, pp. 597-631.
2 K. Kummerer and P. Krauss, *Gewässerschutz, Wasser, Abwasser*, 114 (1990) 109–136.
3 L. Huber and H. Baumung, *Water Sci. Technol., 20 (1988) 19.*
4 W.M.G.M. van Loon, J.J. Boon and B. de Groot, *J. Anal. Appl. Pyrol., 20* (1991) 275–302.
5 D. Budin and L. Susa, *Holzforschung, 36 (1982) 17-22.*
6 P.L. Dubin (Editor), *Aqueous Size-Exclusion Chromatography (Journal of Chromatography Library,* Vol. 40), Elsevier, Amsterdam, 1988.
7 H.G. Barth, *J. Chromatogr. Sci.*, 18 (1980) 409–429.
8 E. Pfannkoch, K.C. Lu, F.E. Regnier and H.G. Barth, *J. Chromatogr. Sci., 18 (1980) 430-441.*
9 T. Vartiainen, A. Liimatainen and P. Kauranen, *Sci. Total Environ., 62 (1987) 75-84.*
10 K. Lindström and F. Osterberg, *Holzforschung, 38 (1984) 201.*
11 F. Osterberg and K. Lindström, *Holzforschung, 39 (1985) 149.*
12 J.J. Boon, in A. Chesson and E.R. Ørskov (Editors), *Physico-Chemical Characterisation of Plant Residues for Industrial and Feed Use,* Elsevier Applied Science, London, 1989, pp. 25-49.
13 J.J. Boon, A.D. Pouwels and G.B. Eijkel, *Biochem. Soc. Trans.*, 15 (1987) 170.
14 W.M.G.M. van Loon, A.D. Pouwels, P. Veenendaal and J.J. Boon, *Int. J. Environ. Anal. Chem., 38 (1990)* 255–264.
15 A.D. Pouwels and J. J. Boon, *J. Wood Chem. Technol., 7 (1987) 197.*
16 N. Dunlop-Jones and L.H. Allen, *Tappi J., 2 (1988)* 109.
17 N.G. Lewis and W.Q. Yean, *J. Chromatogr., 331 (1985) 419-424.*
18 K.K. Unger, *Porous Silica,* Elsevier, Amsterdam, 1979, pp. 130–141.
19 S. Mori, *Anal. Chem., 61 (1989) 530-534.*
20 R.C. Weast (Editor), *Handbook of Chemistry and Physics,* 56th ed., CRC Press, Cleveland, OH, 1975, p. D-150.
21 G.R. Aiken, D.M. McKnight and R.L. Wershaw (Editors), *Humic Substances in Soil, Sediment, and Water,* Wiley, New York, 1985, pp. 477-492.
22 G.L. Amy, M.R. Collins, C.J. Kuo and P.H. King, *Proc. AWWA Annu.* Conf., (1985) 1347-1361.
23 P.R. Gupta and J.L. McCarthy, *Macromolecules,* 1 *(1968) 236.*
24 H. Hassi, P. Tikka and E. Sjostrom, *Tappi (Proc. Int. Sulphite Pulping Conf.)*, (1982) 165–170.
25 D.A.I. Goring, in K.V. Sarkanen and C.H. Ludwig (Editors), *Lignins*, Wiley, New York, 1971, pp. 695-768.