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AQUEOUS SIZE EXCLUSION CHROMATOGRAPHY OF INDUSTRIAL LIGNINS

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SUMMARY

Aqueous size exclusion chromatography of lignin in effluents from the pulp industry was studied using several mobile phases at pH 8.9–12.0 and an organic semi-rigid high-performance gel (TSK PW type). The calibration and resolution calculations were based on the elution of sodium polystyrenesulphonates. The plate counts and plate heights of the columns were also determined. The best results were obtained by using as eluent a low-ionic-strength bicarbonate buffer, pH 10.5, with addition of polyethylene glycol. Molecular weights obtained for spent bleaching liquor lignins and lignosulphonate were compared with results obtained with other chromatographic systems. The distribution curves were symmetrical and narrow. The chromatograms of spent E1-stage bleaching liquor obtained with the system described and with a Bio-Gel P-60 column were very similar, but differed markedly from that obtained with a Sephadex LH-60 column.

INTRODUCTION

Little information is available on the molecular weight distribution (MWD) of lignin in the effluents from pulp bleaching. Aqueous size exclusion chromatography (SEC) has previously been used by Stenlund[1] and Forss *et al.*[2] for analysis of lignosulphonates and kraft lignin on Sephadex G gel. The same gel has also been used with 0.1 *M* NaOH by Sarkanen *et al.*[3,4]. Mixtures of water with dioxane have been used by several authors[5–8] for lignin-related materials on Sephadex G and LH gels.

The acidic groups of lignins dissociate at high pH and the molecules are thus polyelectrolytes. This fact greatly influences their behaviour in aqueous SEC. Difficulties arise from the functional heterogeneity of lignin from spent bleaching liquors. The fractionation may be affected by expansion of the molecules, ion exclusion, ion inclusion, ion exchange and adsorption[1,9]. The expansion of polyelectrolytes in solutions of low ionic strength is due to repulsion between the charges of the molecule and extensive solvation. As a consequence the molecules are excluded from the gel. If the gel and the sample have the same charge, the sample molecules are excluded

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from the pores of the gel (ion exclusion). This may happen in the analysis of lignins with TSK PW gel which is known to contain small amounts of carboxylic groups[10]. Ion exchange takes place if the charge of the gel is opposite to that of the sample. Ion inclusion means that the concentration of small electrolytes in the pores increases if there are polyelectrolytes with similar charge present which cannot enter the pores[1]. This effect leads to delayed elution of small molecules of the sample. Usually ionic interactions can be diminished by adding simple electrolytes to the mobile phase[9].

Adsorption can arise from hydrogen bonding or hydrophobic interactions[9]. Hydrogen bonding may be avoided by adding into the mobile phase some compound (like polyethylene glycol (PEG) which forms hydrogen bonds and saturates the adsorption sites[9,11–13]. An aromatic ring system is sufficiently hydrophobic for reversed-phase adsorption even with hydrophilic supports[14]. The hydrophobic interactions between the gel and the sample may be avoided by using low-ionic-strength mobile phases[9]. In the SEC of polyelectrolytes, however, the eluent must contain sufficient salt to prevent the expansion and ionic interactions of the molecules[15]. It was reported that an ionic strength of 0.01 is sufficient for analysis of polystyrenesulphonates (PSSs)[10]. The polarity of the mobile phase should be such that the gel swells in it but there is no adsorption[13].

Most Finnish pulp mills discharge into inland waters which often also serve as reservoirs of potable water. In order to be able to predict the effects of such discharges on the quality of surface water, more information on the physicochemical characteristics of such organic matter is needed. In this paper we present results of a study of the molecular weight distribution of ligninous material from spent pulping liquors. A high-performance SEC system was developed by optimizing the eluent pH and ionic strength. The performance of the system was estimated using the following parameters: correlation factor of the calibration line, resolution factors, distribution coefficients of model compounds, plate number and plate height.

EXPERIMENTAL

Columns and apparatus

The exclusion limits of the high-performance columns used (TSK G4000PW and G3000PW; Toyo Soda Manufacturing Co., Tokyo, Japan) for polyethylene glycols were 3×10^5 and 5×10^4, respectively[16]. The particle sizes were 10–13 μm. In the course of the experimental work it was found that the G2000PW column adsorbed anionic material and could not therefore be used. Two combinations of columns were used: (1) G4000PW + 2 × G3000PW; (s) G4000PW + G3000PW (see Table IV). The purpose of the second set was to increase the linearity of calibration in the low-molecular-weight range. The dimensions of the Bio-Gel P-60 (Bio-Rad Labs., Richmond, CA, U.S.A.) and Sephadex LH-60 columns (Pharmacia, Uppsala, Sweden) were 100 × 1 cm I.D.

The liquid chromatograph consisted of a Micromeritics 750 solvent-delivery system, 786 variable wavelength UV–visible detector, 771 refractive index (RI) detector (Micromeritics Instrument Corp., Norcross, GA, U.S.A.) and a Rheodyne 7125 injector with a 20-μl loop (Rheodyne, Berkeley, CA, U.S.A.). The PSSs were detected at 262 nm and the other compounds at 280 nm. Ethylene glycol was monitored with the RI detector.

TABLE I

MOBILE PHASES USED

I = Ionic strength.

	Mobile phase	*pH*	*I*
a	Distilled deionized water	5.6	0.00
b	NaOH (0.01 *M*)	12.0	0.01
c	Buffer d–dioxane (4:1, v/v)	10.6	0.03
d	$NaHCO_3$ (0.025 *M*)–NaOH buffer[17]	10.5	0.04
e	Buffer d with 0.5 g/l of PEG (MW 6000)	10.5	0.04
f	H_3BO_3 (0.05 *M*)–NaOH–KCl buffer[17]	8.9	0.12
g	Na_2CO_3 (0.04 *M*)–NaOH*	12.0	0.12
h	Buffer d with 0.1 *M* sodium acetate	10.6	0.14
i	Na_2CO_3 (0.04 *M*)–LiCl (0.54 *M*)–NaOH*	12.0	0.67

* pH was adjusted with 0.1 *M* NaOH to the value indicated.

Mobile phase

The mobile phases tested with TSK PW columns are listed in Table I. The flow-rate was 1.00 ml/min corresponding to a linear velocity of 1 mm/sec. Polyethylene glycol (pract.) was from Fluka (Buchs, Switzerland) and dioxane (zur Analyse) from E. Merck (Darmstadt, F.R.G.). The eluents used with Bio-Gel P-60 and Sephadex LH-60 columns were 25 m*M* Tris–HCl buffer with 0.1 *M* NaCl (pH 8.0), and 1% (v/v) of acetic acid in dimethylformamide (DMF) (E. Merck)[17], respectively. The flow-rates for Bio-Gel and Sephadex columns were 5.9 and 5.1 ml/h respectively.

Chemicals

PSSs or polystyrenes (PSs) of narrow molecular weight distribution (Pressure Chemicals, Pittsburg, PA, U.S.A.) were used as molecular weight reference compounds. The molecular weights of the PSSs were 65,000, 31,000, 16,000, 6500, 4000 and 1600 and of the PSs were 50,000, 17,500, 4000, 2200 and 800. The polydispersity stated by the manufacturer was 1.10 for PSSs except for PSS 1600 (1.25) and was 1.06 for PSs except for PS 800 (1.30). They were used at concentrations of 0.1–1% (w/v).

The low-molecular-weight compounds used were 2-guaiacoxy-3-(3,4-dimethoxyphenyl)-3-hydroxypropionic acid (I), a gift from K. Lundquist (Chalmers Tekniska Högskola, Gothenburg, Sweden), vanillic acid (II) (Fluka), vanillin (III) (BDH, Poole, U.K.) and acetone (IV) (E. Merck). The void volume, V_0, and total permeation volumes, V_t, were determined with dextran blue (Pharmacia) and ethylene glycol (E. Merck) in water solutions and with PS 50,000 and acetone in DMF.

The O- and E1-stage bleaching liquors were from industrial bleaching (OCE-sequence; O = oxygen stage; C = chlorine stage; E = alkali stage) of softwood pulp. The ultrafiltration was performed with an Amicon DM5 filter (Amicon Corp., Danvers, MA, U.S.A.) with a nominal cut-off of 5000 dalton. NH_4-Peritan was a commercial lignosulphonate (Norcem, Oslo, Norway).

THEORETICAL

To compare different SEC systems the following calculations are required:

(1) SEC is based on a linear correlation between the logarithm of the molecular weight and the elution volume. The linearity of the calibration can be evaluated using the correlation factors of the calibration lines. The elution volume must be converted into the partition coefficient, K_d, in order to compare different column sizes.

(2) Resolution describes the ability of the column to separate molecules of different sizes from each other. The difference in the elution volumes and the peak widths affect the resolution. Resolution, R_s, in SEC can be expressed[19] by

$$R_s = \frac{\ln (M_2/M_1)}{2D_2(\sigma_1 + \sigma_2)} \quad (1)$$

where σ_1 and σ_2 are the standard deviations of the distribution of two compounds of molecular weights M_1 and M_2, and D_2 is the slope of the calibration line.

Since there usually are no separate peaks in the chromatograms it is useful to calculate the resolution over the whole area of fractionation, R_{sp}, by dividing R_s by $\ln (M_2/M_1)$[19]. The packing resolution factor, R_{sp}*, may be used to compare columns of different sizes[19]; $R_{sp}^* = R_{sp}/\sqrt{L}$, where L is the length of the column.

(3) The band broadening in the column can be expressed in terms of the number of theoretical plates, N. The latter is measured using a completely permeating monodisperse solute. The height of a theoretical plate, H, is L/N.

RESULTS AND DISCUSSION

Direct SEC analysis in water has the advantage that sample losses or modification of the sample due to derivatization or evaporation are avoided. Most lignins are soluble only at alkaline pH values, where most SEC gels are unstable. Silica-based gels begin to dissolve above pH 7[20] and the expected life of Sephadex G gel in 0.5 *M* NaOH is short. The bleaching liquors studied here were soluble at pH 9. Lignosulphonates are water-soluble and can readily be chromatographed in neutral salt solution[1,2].

Of the mobile phases of similar ionic strength (f, g and h; Table II), the best packing resolution factor, R_{sp}*, was obtained at pH 10.6 (h). This pH should be sufficiently high for dissociation of the compounds in spent bleaching liquors. It will be seen that ionic strength is much more important than pH in the fractionation of the samples.

The most linear calibration at pH 10.5–10.6 (c, d, e, h) was obtained at the lowest ionic strength (c, $R = 0.999$), but the best packing resolution factor was found at the highest ionic strength (h, Table II). The resolution was not calculated for eluent a (distilled water) because there was no size fractionation (Fig. 1) and the peaks were strongly skewed. The resolution factors in Table II were calculated using the slopes (D_2, eqn. 1) of the regression lines. However, as it is seen from Fig. 1, the plot of log MW *vs.* K_d was not linear for the high ionic strength eluents (f, g, h). PSS 1600 was clearly retained in these mobile phases, indicating the onset of adsorption. At the highest ionic strength (0.67, eluent i) all the PSSs were adsorbed by the gel and no calibration line was obtained. Increasing adsorption with increasing ionic strength in aqueous SEC has been reported previously[14,15]. Omorodion *et al.*[11] used a porous

TABLE II

RESOLUTION OF PSS IN DIFFERENT MOBILE PHASE

Resolution, R_s, was calculated from the peaks of PSS 16,000 and 4000. I = Ionic strength; R = correlation factor. See text for definitions. Conditions as in Table I.

Mobile phase	R_s	R_{sp}	$R_{sp}^{\star}$	I	R
b	0.58	0.96	1.24	0.01	0.998
c	0.59	0.98	1.27	0.03	0.999
d	1.01	1.68	1.77	0.04	0.971
e	0.71	1.17	1.52	0.04	0.996
f	0.79	1.31	1.38	0.12	0.980
g	0.84	1.40	1.80	0.12	0.952
h	1.16	1.92	2.48	0.14	0.957

glass packing and found that high-molecular-weight (MW > 88,000) PSSs were retarded when the ionic strength was increased from 0.01 to 0.5 (NaCl), whereas there was no change in the elution volume of PSSs 31,000. The resolution was strongly reduced at ionic strengths higher than 0.05[10]. In this study it was found that the K_d values of all the PSSs increased at high ionic strength, leading to increased apparent resolution when the linear regression line of all the data points (Fig. 1) was used for the calculation. The resolution at high ionic strengths would have been lower if only the linear part of the calibration plot had been used. The increase in the elution volumes of PSSs at higher ionic strengths may partially be due to diminished chain dimensions[1,2,9,11,21]. Spatorico and Beyer[22], however, noticed no significant change in the elution volumes of PSSs on a porous glass packing when the ionic strength was increased from 0.2 to 0.8 M (Na_2SO_4).

The observed adsorption was studied using low-molecular-weight compounds I–IV. The distribution coefficients of these compounds are presented in Table III. A

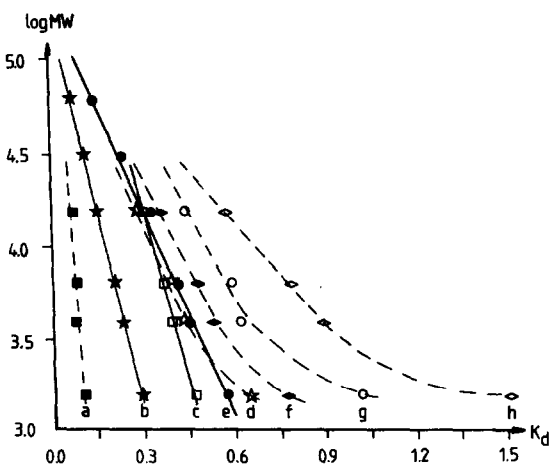

Fig. 1. Calibration plots for PSSs in different mobile phases (for composition of the mobile phases see Table I).

TABLE III

DISTRIBUTION COEFFICIENTS OF MODEL COMPOUNDS OF LOW MOLECULAR WEIGHT

I = 2-Guiacoxy-3-(3,4-dimethoxyphenyl)-3-hydroxypropionic acid; II = vanillic acid; III = vanillin; IV = acetone. $K_d = (V_R - V_0)/(V_t - V_0)$, where V_0 and V_t are the void volume and total permeation volume of the column.

Mobile phase	*Compounds*			
	I	*II*	*III*	*IV*
a	–	–	–	1.29
b	0.67	0.42	0.72	1.39
c	0.83	0.65	0.93	1.24
d	1.44	0.70	1.61	1.40
e	1.17	0.65	1.33	1.36
f	1.16	0.87	1.93	1.42
g	1.41	0.66	0.93	1.24
h	1.76	0.81	2.06	1.40
i	–	0.80	2.09	1.39

value $K_d > 1$ indicates adsorption, but one should remember that the K_d values are relative to ethylene glycol which was used to determine the total permeation volume, V_t. The values of K_d would be lower if acetone had been used as a measure of V_t.

An increase in the ionic strength of the eluent seemed to retard compounds I and III (Tables I and III). We therefore conclude that ion inclusion or ion exchange was not causing the adsorption. Ion exchange was unlikely since the gel does not contain cationic groups and compounds I and III were present as anions in the solution.

To study the role of hydrogen bonding, PEG was added to the mobile phase d. It was found that the K_d values of compounds I and III decreased when PEG was used (Table III, mobile phases d and e). One may conclude that the adsorption was at least partly due to hydrogen bonding. There was also hydrophobic adsorption since the addition of dioxane to the mobile phase lowered the K_d values for compounds I and III (compare mobile phase c to d, Table III). This confirms the result reported by Dubin *et al.*[23] who found that the elution volumes of *n*-alcohols on TSK PW columns decreased when 50% methanol was used as eluent instead of 0.1 *M* NaCl. They also concluded that the gel probably contains anionic sites since the apparent MW values of cationic polymers were higher at higher ionic strength[23].

Vanillic acid (compound II) was eluted too early relative to its MW in each of the tested mobile phases. Ion exclusion was not the reason for this since compounds I and III which also are anions were not similarly eluted (Table III). This phenomenon might be connected to the fact that this compound is both a carboxylic acid and a phenol, since ferulic acid was eluted similarly (data not shown), and may thus reflect some intermolecular electrostatic effect. The apparent molecular weight of vanillic acid was about 1000 (true value 168) and thus much more than the MW of an association dimer.

The number of theoretical plates, *N*, and the plate heights, *H*, are presented in Table IV. The experimentally obtained values were higher than expected from the

TABLE IV

PLATE NUMBERS, N, AND PLATE HEIGHTS, H

$N = 5.54\ (V_R/W)^2$, where W = peak width at half-height, and $H = L/N$, where L = length of the column.

Mobile phase	*N*	*H*	*Number of columns*
a	8780	0.10	3
b	7710	0.08	2
c	7370	0.08	2
d	9510	0.09	3
e	7400	0.08	2
f	8260	0.11	3
g	7480	0.08	2
h	7490	0.08	2
i	7340	0.08	2

data provided by the manufacturer (N = 3000 for G4000PW and N = 5000 for G3000PW)[16]. The decrease in N when two columns were used instead of three was due to the fact that the plate count of a G4000PW column is less than that of a G3000PW column[16].

The plate heights were in all cases smaller with only two columns (Table IV) indicating smaller solute dispersion in the column. By comparing Tables II and IV it can be concluded that N or H does not provide a good measure of the separation efficiency; the increase or decrease in R_{sp}* cannot be seen from N and H. They can, however, be used to measure the extent of extra-column effects, such as the existence of dead volume in the connections of the chromatograph, or to see the possible change in the gel when the mobile phase is changed.

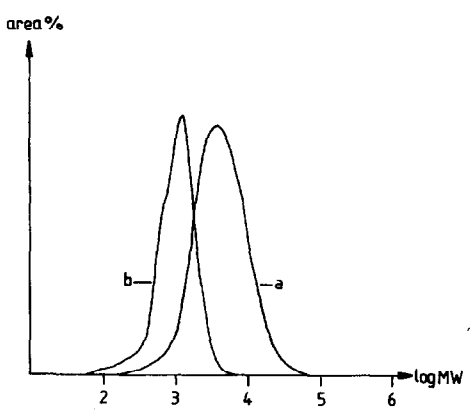

Fig. 2. Molecular weight distributions of two fractions of ultrafiltered spent bleaching liquor from the O-stage (softwood kraft pulp, OCE-bleaching). Mobile phase e, TSK G4000PW + G3000PW columns. a, MW > 5000 g/mol; b, MW < 5000 g/mol.

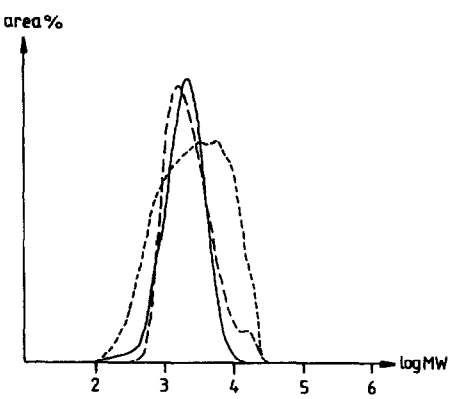

Fig. 3. Molecular weight distributions of E1-stage bleaching liquor from oxygen bleaching of softwood kraft pulp (OCE-bleaching). ——, TSK G4000PW + G3000PW column, mobile phase e; ----, Sephadex LH-60 column, mobile phase 1% (v/v) acetic acid in DMF; – – –, Bio-Gel P-60 column, mobile phase Tris–HCl buffer (25 mM) + 0.1 M NaCl.

Applications

Mobile phase e provided the optimum compromise between the linearity of the calibration (Fig. 1) and the resolution (Table II) and was therefore chosen for the analysis of spent bleaching liquors and lignosulphonate. The method described here proved impractical for the analysis of kraft lignin because of strong adsorption of the sample (results not shown) and poor solubility at pH 10.5. This may be a consequence of the fact that in kraft lignin there are more phenolic and less acidic groups than in bleaching liquor lignins, leading to less dissociation of kraft lignin at this pH. To analyse this kind of material in water solution, 0.1 *M* NaOH should be used as eluent[2–4].

The molecular weight distributions of ultrafiltered bleaching liquor fractions from the O-stage of OCE-bleaching of softwood kraft pulp in mobile phase e are shown in Fig. 2. The weight average molecular weights, $\bar{M}_w$, of 7000 and 2400, and the number average molecular weights, $\bar{M}_n$, of 1100 and 670 were obtained for the retentate (MW > 5000) and for the permeate (MW < 5000), respectively.

A set of chromatograms for E1-stage spent liquor from OCE-bleaching of softwood pulp in different chromatographic systems is shown in Fig. 3. The corresponding average molecular weights and polydispersities are presented in Table V. The lowest values were obtained using TSK columns. The weight average MW and polydispersity were much higher when Sephadex LH-60 was used than with the other systems. The analysis time was 18 min for TSK PW columns, and 18 h for the others. The apparent MW of the UV-absorbing material in E1-stage spent liquor from oxygen bleaching (OCE) was slightly less than that reported for chlorine bleaching (CEH)[24], but similar to that reported previously for oxygen bleaching of softwood pulp[25]. Pfister and Sjöström[25] fractionated OCE-bleaching liquors by ultrafiltration and found that *ca.* 10% of the lignin of the O-stage effluent was in the MW < 1000 permeate, but almost all of the lignin of the E1-stage effluent was in the fractions of MW > 1000.

Fig. 4 shows the MWD of NH_4-Peritan (lignosulphonate) in mobile phase e. The calculated average molecular weights were $\bar{M}_n$ = 2600 and $\bar{M}_w$ = 6700, and the polydispersity 2.57. These values are much lower than that reported by Herrick *et al.*[26]; $\bar{M}_n$ = 8100, $\bar{M}_w$ = 504,000 (SEC), but somewhat higher than that reported for quaternary ammonium salt of lignosulphonate (precipitated with Hyamine 10-X, determined by vapour pressure osmometry), $\bar{M}_n$ = 1700[27].

TABLE V

AVERAGE MOLECULAR WEIGHTS OF E1-STAGE SPENT LIQUOR FROM OCE-BLEACHING DETERMINED IN DIFFERENT CHROMATOGRAPHIC SYSTEMS

$\bar{M}_n$ = Number average molecular weight; $\bar{M}_w$ = weight average molecular weight; PD = polydispersity ($\bar{M}_w/\bar{M}_n$).

System	$\bar{M}_n$	$\bar{M}_w$	*PD*
TSK PW*	1600	2700	1.68
Bio-Gel P-60	2000	3500	1.80
Sephadex LH-60	1700	4900	2.94

* Mobile phase e, TSK G4000PW + G3000PW columns.

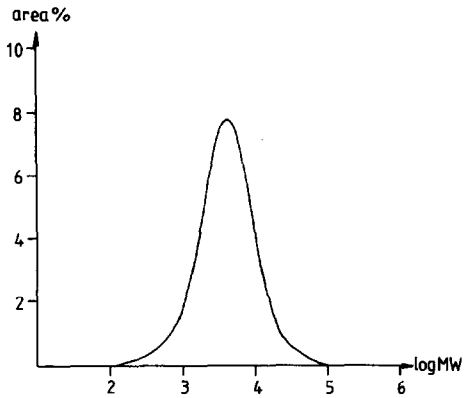

Fig. 4. Molecular weight distribution of a commercial lignosulphonate preparation (NH_4-Peritan). Mobile phase e, columns as in Fig. 2.

The error in the MWs determined with the present method was estimated using PSS standards to be ± 12%. This includes the error caused by irregular flow, but not that caused by the different structures of the standards and samples. The molecular weights obtained by SEC are relative to compounds used for the calibration and depend on the conditions, especially in aqueous SEC. It would be optimal to calibrate SEC columns with reference compounds of the same structure as the material to be fractionated. In the case of industrial lignins this is not possible since the material is heterogeneous, containing acids, phenols and neutral compounds and the composition of spent liquors is not constant. In this study, polystyrenesulphonates were chosen as reference compounds because they are aromatic compounds and thus absorb UV light, and are commercially available within a suitable MW range. Sarkanen *et al.*[4] used Sephadex G gel in 0.1 *M* NaOH and reported that the calibration lines obtained with kraft lignin fractions and PSSs were parallel, and that the MWs of lignin fractions were larger by a factor of 1.7 than those of PSSs at the same elution volume. It is thus possible that the MWs determined with this method are underestimates.

The formation of association complexes often disturbs SEC of lignins. Association occurs especially when the pH of the mobile phase is low, or when the concentration or molecular weight is high[3,4]. Multimodal elution curves are often caused by association[3]. We believe that the chromatographic system described here was free from association effects since the elution curves were monomodal and approximately Gaussian in shape at every pH, the concentrations were low (less than 5 mg/ml) and the molecular weights were relatively low.

CONCLUSIONS

Lignosulphonates and lignin from bleaching liquors can be fractionated with aqueous HPSEC as described, but the fractionation of material with MW less than 500 may be disturbed by adsorption. If the material to be analysed contains mainly molecules larger than 3000 g/mol, the ionic strength of the mobile phase can be increased to 0.14 to improve resolution while maintaining good linearity of the cal-

ibration. The chromatographic system described may be valuable in studying the effects of bleaching waste waters on the environment, and the biodegradation of industrial lignins.

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