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Journal of Chromatography A, 942 (2002) 191–199

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Molecular weight determination of lignosulfonates by size-exclusion chromatography and multi-angle laser light scattering

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Received 9 May 2001; received in revised form 8 October 2001; accepted 8 October 2001

Abstract

A lignosulfonate sample was fractionated according to the solubility in ethanol–water. The fractions were analysed by aqueous size exclusion chromatography (SEC) combined with in-line multi-angle laser light scattering (MALLS), and by static MALLS. Satisfactory SEC results were obtained with aqueous phosphate buffer containing DMSO and SDS. The refractive index increment (dn/dc) varied from 0.186 to 0.205 ml/g, depending on M_w and the degree of sulfonation. The second viral coefficient (A_2) was 7×10^{-3} ml mol/g^2. The weight-average molecular weight (M_w) of the fractions varied from 4600 up to 398 000 g/mol, and the polydispersity (M_w/M_n) varied between 1.3 and 3.5.

Keywords: Molecular mass determination; Multi-angle laser light scattering; Lignosulfonates

1. Introduction

Lignosulfonates are produced in the sulfite pulping process as a by-product in the production of cellulose. In contrast to native lignin, the lignosulfonates are water-soluble, due to the fragmentation and the introduction of sulfonate groups. Both lignosulfonates and lignins have very compact structures as demonstrated by low intrinsic viscosities, typically 6–15 ml/g [1], even for high molecular weight samples ($M_w>100\,000$). Commercial lignosulfonates are known to have broad molecular weight distributions [2], and the degree of sulfonation varies from 0.4 to 0.7 sulfonate groups per phenylpropane residue [3]. Lignosulfonates are used in several industrial fields, mainly as dispersants and binders. Many of the functional properties depend on the molecular weight, and a method for convenient and accurate molecular weight determination is therefore needed.

The molecular weight of lignosulfonates and lignosulfonate fractions has been determined by a variety of methods. These include conventional (wide-angle) light scattering [1,2,4], low angle laser light scattering [5], sedimentation equilibrium [6] and sedimentation velocity [4,7]. Gel filtration on Sephadex columns has been used to provide a

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0021-9673/02/$ – see front matter
PII: S0021-9673(01)01377-2

qualitative description of the molecular weight distribution by comparing the elution profile to non-lignosulfonate standards [8]. By including commercially available molecular weight standards such as poly(styrene sulfonates) (PSS) or pullulans this method has allowed quantitative estimates of the molecular weight distribution and molecular weight averages of lignosulfonates. The major drawback in using polymers other than lignosulfonates as standards is the difference in hydrodynamic volume due to different shapes and extensions. This has been demonstrated for Kraft lignins, where the molecular weights were 1.7 times higher than those of the PSS standards at the same elution volume [9]. The gel filtration method has been improved by collecting lignosulfonate fractions and subsequently determining their molecular weights by light scattering or analytical ultracentrifugation [10,11]. Such fractions provide more relevant standards in subsequent experiments, although they are not monodisperse and are not available for other investigators. Several investigators have introduced aqueous HPLC (SEC) instead of gel filtration due to the generally improved chromatographic resolution and much shorter analysis times [9,12–15]. However, the problem using non-lignosulfonate standards has persisted in most of these investigations, except for one case [13], where lignosulfonates that had been independently characterised by analytical ultracentrifugation were used for calibration. Non-SEC phenomena like adsorption and ion exclusion must be considered since lignosulfonates are both hydrophobic (aromatic) and ionic.

A major improvement in the field of molecular weight determination is the on-line combination of SEC and light scattering (LALLS or MALLS). This approach has routinely and successfully been applied to a variety of polymers and biopolymers. In contrast, reports on lignosulfonates have not been presented in the scientific literature. A technical note from an instrument supplier is available [16].

In light scattering the observed zero-angle ($\theta = 0$) Rayleigh factor (R_0) is related to the weight-average molecular weight through the standard equations:

$$Kc/R_0 = 1/M_w + 2A_2c \quad (1)$$

$$K = 4\pi^2 n_0^2 (dn/dc)^2 N_A^{-1} \lambda_0^{-4} \quad (2)$$

(dn/dc) is the refractive index increment (which has to be known from independent measurements), n_0 is the refractive index of the solvent, N_A is Avogadro's number, λ_0 is the wavelength of the incident light (in vacuo), and A_2 is the second viral coefficient. The factor 4 in Eq. (2) applies to vertically polarised incident light. For each elution slice (i) in SEC the concentration (c_i) is obtained from a concentration sensitive detector, usually a refractive index detector or UV detector, and R_0 is obtained from the MALLS (after extrapolation to zero angle) or a LALLS detector ($\theta \approx 0$). $M_{w,i}$ is then calculated according to Eq. (1). A_2 must be known from independent (batch) light scattering measurements, unless the sample concentration is kept so low that $A_2c \ll 1/M_w$. The radius of gyration (R_G) may in principle be obtained from the angular dependence of R_θ, provided $R_G > \lambda/20$. The latter is normally not obtained due to the small size of the lignosulfonate molecules. M_n, M_w and M_z (and the corresponding mean square radius moments) can in principle be calculated by considering each elution slice as monodisperse [17].

Lignosulfonates and lignins exhibit fluorescence, which is detectable in light scattering instruments even if the wavelength of the incident light is well above the absorption region [2,18]. Introduction of narrow band-pass filters is needed to eliminate this effect [5]. Depolarisation due to optical anisotropy may further influence light scattering measurements of low molecular weight samples [4]. In the present investigation samples with relatively high molecular weights are analysed, and depolarisation is therefore not considered [19].

Parameters obtained from SEC combined with light scattering do not only depend on the macromolecular properties alone. The instrument configuration, the experimental conditions (columns, buffer/salt, temperature, flow-rate, in-line filters), as well as the sample preparation, may influence the final results. Correct results on an absolute basis further require that processing parameters (dn/dc, A_2 etc.) and detector responses (calibration factors) are correctly determined. Although light scattering detectors in principle provide absolute molecular weights without the use of calibration substances, the access to relevant standards remains crucial in order to check the performance of the system [20].

In this work the starting material was an industrial

sample of lignosulfonate with a broad molecular weight distribution. The first aim was to produce lignosulfonate fractions with narrower molecular weight distributions. A fractionation procedure where lignosulfonates were separated according to molecular weight with extraction of ethanol of different concentration was performed [21]. The method was scaled up to provide larger amounts of each fraction. These were then analysed by SEC–MALLS.

2. Experimental

2.1. Materials

An ultrafiltered and ion-exchanged high molecular weight Na-lignosulfonate sample (spruce, sulfite process) was obtained from Borregaard LignoTech, Norway. Essentially monodisperse PSS standards (Fluka), polydisperse PSS (Polysciences) and a polysaccharide (Pullulan P-137, Hayashibara Biochemical Laboratories) were analysed for comparison and testing of the method.

2.2. Fractionation procedure

First, 60 g Na-lignosulfonate was dissolved in 1000 ml water and ion-exchanged to the corresponding acid form (lignosulfonic acid) with Amberlite (IR-120, Fluka). The mixture was stirred for 2 h and the lignosulfonic acid solution was removed by decanting. The Amberlite was then rinsed thoroughly and regenerated. The procedure was repeated three times until pH of the solution was 1.3. The sample was further evaporated back to a volume of 1000 ml. $BaCO_3$ was added to the solution under stirring to pH 3 to obtain Ba-lignosulfonate. The sample was subsequently centrifuged at 4000 rpm for 10 min to remove precipitated $BaSO_4$ and evaporated (rotavapor) to a volume 300 ml. The Ba-lignosulfonate solution was then mixed with 130 g milled cellulose (Borregaard ChemCell) and suspended in 1000 ml 96% ethanol [21]. A column (11 cm I.D., 30 cm high) was packed with milled cellulose suspended in ethanol (96%). The Ba-lignosulfonate–cellulose mixture was introduced onto the cellulose column. The column was eluted stepwise with ethanol–water mixtures containing 96, 70, 60, 55, 50, 45 and 40% (v/v) ethanol. Six fractions (F-70, F-60, F-55, F-50, F-45, F-40) were obtained at the corresponding ethanol concentrations. The fractions were evaporated, ion-exchanged back to the Na^+ form, and dried at 60°C.

2.3. Determination of dn/dc

The refractive index increment (dn/dc) was determined using a Shimadzu RID-10A RI-detector calibrated with NaCl (dn/dc = 0.174 at 633 nm [22]). The samples (0.2–2.0 mg/ml) were filtered (Millex HA, 0.45 μm) and injected directly into the RI-detector with a syringe-pump (Razel).

2.4. Static (batch mode/total intensity) multi-angle laser light scattering

Analyses were performed on a DAWN-F multi-angle laser light scattering detector (Wyatt Technologies). Detectors 15, 13, 11, 9 and 7 were equipped with narrow band-pass filters (Wyatt Technologies) to eliminate fluorescence. Measurements were performed at room temperature using simple glass vials. The samples were filtered through 0.45-μm syringe filters (Acrodisc, GHP) prior to analysis. The solvent was the SEC (phosphate–DMSO–SDS) mobile phase described below. M_w and A_2 were calculated from a conventional Zimm plot on the basis of a concentration series.

2.5. Size exclusion chromatography–multiangle laser light scattering (SEC–MALLS)

SEC was performed using a HPLC pump (Perkin-Elmer, Series 200) equipped with an autosampler (Perkin-Elmer, Series 200). The flow-rate was 1.00 ml/min, and the injection volume was 200 μl (concentration 2–5 mg/ml). The instrument set-up consisted of a SEC-column (Jordi Glucose — DVB, 10^4 Å pore size, 500×10 mm) in an oven (Eppendorf) at a temperature of 60°C, combined with a DAWN-F MALLS detector (equipped with fluorescence filters as described above) followed by a RI detector (Shimadzu RID-10A). The system was also equipped with a guard column (TSK PWXL, 300 Å, 7 μm). Data acquisition and molecular weight calcu-

lations were performed using the ASTRA software, Version 4.70.07 (Wyatt Technologies). The mobile phase was prepared by mixing 80.9 g DMSO (HPLC grade, Aldrich) with 800.0 g water (Milli-Q). 10.72 g $Na_2HPO_4 \cdot H_2O$ (reagent grade, Aldrich) was added and pH was adjusted to 10.50 with NaOH (reagent grade, Aldrich). Then, 0.8 g of sodium dodecyl sulfate (SDS) (reagent grade, Aldrich) was added. The solution was stirred under vacuum for 30 min between each step. The mobile phase was finally filtered through a 0.22-μm filter (Millipore, type HA). The samples were prepared by dissolving between 20 and 50 mg dry lignosulfonate (dried at 105°C for 1 h) in 10 ml mobile phase followed by filtering through 0.45-μm syringe filters (Acrodisc, GHP) directly into glass vials.

In an alternative series of SEC–MALLS measurements the analyses were performed with a TSK G3000 PWXL column and an aqueous solvent containing 0.05 *M* Na_2SO_4 and 0.01 *M* EDTA (pH 6.0), with and without 20% acetonitrile.

2.6. Sulfur analysis

The sulfur analyses were performed on a NCS element analyser (Thermo Quest) equipped with a combustion column (1000°C) and a GC column (Säntis Analytical, 990723) for sulfur determination and a steel column (Säntis Analytical, 990720) for carbon determination, respectively. Approximately 1 mg vanadium pentoxide (catalyst) and 1.00 mg sample were wrapped in a tin capsule and injected by an autoinjector. BBOT (2,5-bis(5-*tert.*-butyl-benzoxazol-2-yl)-thiophene) (CE Instruments) was used as standard for sulfur. Sulfanilamide (CE Instruments) was used as standard for carbon.

3. Results and discussion

3.1. Fractionation

Lignosulfonate fractions were obtained by fractional dissolution of precipitated Ba-lignosulfonate from a cellulose column by ethanol–water mixtures with decreasing ethanol contents (96–40%) (Table 1). One may note the high amount of fraction F-45 compared to fraction F-50 and F-40. The results show that at a 45% ethanol concentration about 90% of the Ba-lignosulfonate molecules are soluble.

The degree of sulfonation of each fraction was measured on the basis of C- and S-analyses, and the results are reported in Table 1 as the number of sulfonate groups per C_9 unit. The degree of sulfonation was calculated assuming 95% coniferyl alcohol ($C_{9.95}$) and neglecting possible inorganic sulfur in the fractions. The value increases from 0.39 (F-40) to 0.64 (F-70), indicating that the fractionation (i.e. solubility) is not only governed by the molecular weight, but also by the sulfonate content, which is in agreement with earlier investigations [3].

Table 1
Recoveries, (d*n*/d*c*) values, molecular weight-averages (from SEC–MALLS), and the degrees of sulfonation obtained for unfractionated lignosulfonate and the six lignosulfonate fractions

Fraction	Recovery [g]	d*n*/d*c* [ml/g]	M_n [c] [g/mol]	M_w [g/mol]	M_z [g/mol]	M_w/M_n	$SO_3/C_{9.95}$
Unfractionated	–	0.195[a] 0.192	7200	64 000	–	8.8	–
F-70	14.0	0.186	3200	4600	5600	1.5	0.64
F-60	7.4	0.196	6100	8000	10 000	1.3	0.53
F-55	7.3	0.200	9900	15 000	22 000	1.5	0.49
F-50	4.5	0.204	18 000	34 000	64 000	1.9	0.44
F-45	12.7	0.203	31 000	68 000	139 000	2.3	0.41
F-40	6.1	0.205[b] 0.215	115 000	398 000	–	3.5	0.39

[a] In the presence of SDS.
[b] Dialysed sample.
[c] Obtained with polynomial fit.

3.2. Development of a mobile phase for SEC

An aqueous solvent containing 10% dimethylsulfoxide (DMSO), 0.05 *M* Na_2HPO_4 and 0.1% sodium dodecylsulfate (SDS) buffered to pH 10.5 was used in most of the experiments. This mobile phase was originally developed for analysis of both lignosulfonates and Kraft lignins. SDS was added to prevent absorption of lignosulfonates to the column material (J. Gargulak, personal communication). DMSO was included based on recommendation from the manufacturer of the columns (Jordi Associates). DMSO is a strong polar component and will prevent association of the lignosulfonate molecules [23]. This mobile phase yielded satisfactory SEC results as judged on the basis of sample recoveries, and from the shape of the calibration curves ($\log M$ versus elution volume) obtained by SEC–MALLS (see below).

One sample (F-45) was also analysed by SEC using a TSK G 3000 PWXL column with 0.05 *M* Na_2SO_4–0.01 *M* EDTA (pH 6.0) as mobile phase. In this case partial absorption and excessive tailing were observed. However, apparently normal behaviour was observed upon the addition of 20% acetonitrile (Fig. 5b). Aqueous solvents with added electrolyte (ionic strength 0.1 *M*) and 20% acetonitrile are probably well suited for analysis of most lignosulfonates, but presumably not for lignins.

3.3. Determination of the refractive index increment (dn/dc)

Accurate values of the specific refractive index increment are required in light scattering because (dn/dc) appears in a quadratic form in Eq. (2). In SEC–MALLS also the RI detector signal is inversely proportional to (dn/dc). In result, M_w becomes linearly dependent on (dn/dc) in this case (provided $1/M_w \gg 2A_2c$, which is usually the case in SEC). Results for the unfractionated sample and the six fractions dissolved in the mobile phase (phosphate–SDS–DMSO) are given in Table 1. For the unfractionated sample a value of 0.192 ml/g was obtained. Since the presence of SDS resulted in foaming problems during the measurements the experiment was repeated without SDS. In this case only a marginal increase was observed (0.195 ml/g). Although SDS is surface active and may possibly interact with the lignosulfonates, the results show that SDS may in practice be omitted from the solvent in dn/dc measurements. The remaining measurements were therefore performed without SDS. The results obtained for fractions F-40–F-70 show that dn/dc decreases slightly with decreasing molecular weight and increasing degree of sulfonation. The latter may be ascribed to the lower specific refractivity of sulfonate groups as compared to the phenylpropane units. A similar effect has indeed been observed in sulfated polysaccharides such as carrageenans, where dn/dc decreased with increasing sulfate content [24]. Our values are slightly lower than those obtained for lithium lignosulfonates (0.213 ml/g in pure water and to 0.211 in 1.0 N LiCl) [4].

In one experiment (sample F-40) dn/dc was determined after thorough dialysis against the solvent, thereby obtaining dn/dc at constant chemical potential ($(dn/dc)_\mu$), which is the fundamental value that ideally should be used in all cases. The value of $(dn/dc)_\mu$ turned out to be somewhat higher (0.215 ml/g) than that obtained by direct dissolution without dialysis (0.205). This difference corresponds to a difference in calculated M_w (in SEC–MALLS) of only 5%. For the present purpose this difference in dn/dc was therefore neglected, and dn/dc values obtained for undialysed samples are used in the following.

3.4. Static (total intensity) multi-angle laser light scattering

Fraction F-50 was studied by conventional (static) light scattering to obtain M_w and the second viral coefficient (A_2), as the latter is needed in subsequent SEC–MALLS analyses. The result is presented as a conventional Zimm plot in Fig. 1. A weight-average molecular weight (M_w) of 39 000 and an A_2 value of 7×10^{-3} mol ml/g^2 were obtained using the mobile phase (phosphate–SDS–DMSO) solvent. Such high A_2 values are typically found for polyelectrolytes at medium ionic strengths. As expected no angular dependence of the scattered light could be observed, and the radius of gyration was therefore not obtained.

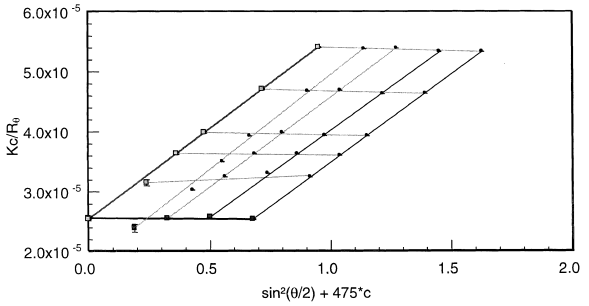

Fig. 1. Zimm plot for fraction F-50.

3.5. SEC–MALLS

The six fractions and the unfractionated sample were analysed on SEC–MALLS with a Jordi Glucose DVB column and the phosphate–SDS–DMSO mobile phase. Fig. 2a shows elution profiles of the six fractions. A progressive shift towards higher elution volumes reflects the decrease in weight average molecular weight (M_w). The areas under the peaks were integrated, and the sample recoveries were calculated. Over 90% recovery was obtained for all samples, indicating that little material adsorbed to the column and that little low molecular weight components eluted in the salt peak. Close to 100% recovery also indicates that the dn/dc values are correct since the integrated RI signal is directly proportional to dn/dc (the proportionality constant was determined separately for the RI detector by calibration with NaCl). For fraction F-70 the recovery was approximately 70%. Fraction F-70 was the first fraction to be collected in the ethanol fractionation step, and it is probable that it contains contamination (sugars etc.) and low MW components that elute in the salt peak.

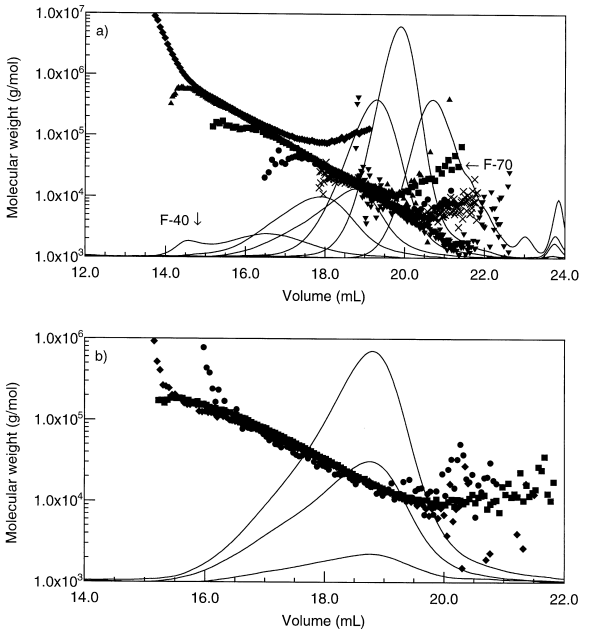

Fig. 2. Calibration plots and elution curves for lignosulfonates. (a) Fraction F-70 (▼), F-60 (×), F-55 (●), F-50 (■), F-45 (▲) and F-40 (♦). (b) Different amount injected of fraction F-50: 0.5 mg/ml (●), 2.0 mg/ml (♦) and 4 mg/ml (■). Injection volume: 0.2 ml.

The calculated plots of $\log M_w$ versus elution volume (calibration plots) are included in Fig. 2a. We obtain basically the same calibration curve for all the fractions. This indicates that the column covers the entire molecular weight range, and that peak broadening does not influence the results. Excessive peak broadening would result in less steep and less continuous calibration curves. It further demonstrates that all fractions belong to a family of polymer chain with the same basic conformation, only differing in the molecular weight. The curves are essentially linear in the elution range 14–21 ml. At lower elution volumes a slight upward curvature is observed in the calibration curves, particularly for fraction F-40. The curvature for F-40 may be due to reduced column resolution near the exclusion limit. The effect for the other fractions may be attributed to some aggregation.

In order to obtain accurate estimates of the number average molecular weight (M_n) and the polydispersity index (M_w/M_n) it is necessary that a calibration curve be correctly assigned across the entire distribution. This is usually not achieved at the low molecular weight tail of the distribution because of a weak light scattering signal. The most appropriate method for estimating M_n across the entire elution interval would probably be to construct a single calibration curve based on all fractions and then recalculate the molecular weight distribution (and corresponding averages) for each fraction [20]. However, this option is not easily performed with the ASTRA software (but is apparently possible in other commercial softwares such as CORONA), and a linear

fit (first order polynomial) was selected for each fraction. For unfractionated lignosulfonate and fraction F-40 the ''aggregate peak'' was integrated with no fit and the main peak with polynomial fit, and a combined molecular weight was calculated. The dependence of the chromatographic behaviour on the amount of injected lignosulfonate was tested by injection of three concentrations of fraction F-50 (0.5, 2.0 and 4.0 mg/ml). The elution profiles and the corresponding calibration curves (Fig. 2b) are slightly shifted towards higher elution volumes with increasing amounts of injected sample, whereas the calculated M_w was independent of the sample amount.

The calculated weight and number average molecular weights and polydispersities (M_w/M_n) are summarised in Table 1. The results demonstrate that such commercial lignosulfonates are extremely polydisperse since fractions with molecular weights ranging from 4600 (F-70) to 398 000 g/mol (F-40) can be obtained. Accordingly, the polydispersity of the unfractionated sample is very high (8.8). The fractions were generally much less polydisperse, especially for the low molecular weight fractions (F-70–F-55). Fraction F-40 had an estimated polydispersity of 3.5, which is partly attributed to presence of large aggregates (corresponding to the upturn in the calibration curve near the exclusion limit), and partly to reduced selectivity in the ethanol precipitation method at very high molecular weights. The fractionation procedure might possibly have been improved using smaller ethanol concentration increments to obtain less polydisperse fractions.

As in the static MALLS experiment, no angular dependence of Kc/R_θ (or R_θ) was observed for any of the fractions, indicating that R_G well below 30 nm ($<\lambda/20$).

Fig. 3 shows the calculated differential molecular weight distributions for each of the six fractions. The distributions partially overlap as also observed directly in the chromatograms. The trend towards more narrow distributions with decreasing average molecular weight is clearly demonstrated. A polynomial fit was chosen for all the fractions to calculate the differential molecular weight distributions. For fraction F-40 this will lead to some underestimation of the high molecular weight molecules.

The importance of including fluorescence filters in the light scattering photometer in the study of lignosulfonates — even when using a laser at 632.8 nm — is clearly demonstrated in Fig. 4. The figure shows the chromatograms obtained with detectors 7–15 (corresponding to 52–132°), where detectors 7, 9, 11, 13 and 15 are equipped with interference filters. In the absence of filters about 50% of the observed signal is due to fluorescence, which would lead to a corresponding overestimation of the molecular weight.

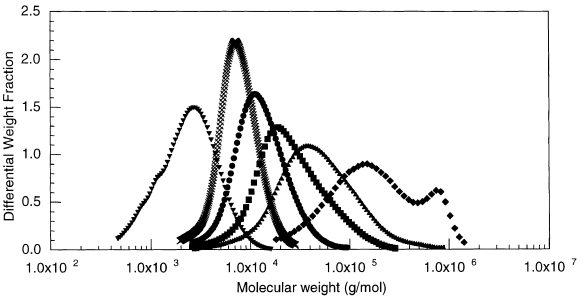

Fig. 3. Calculated differential molecular weight distributions of fractions F-70 (▼), F-60 (×), F-55 (●), F-50 (■), F-45 (▲) and F-40 (♦).

Although SEC–MALLS is in principle an absolute method for molecular weight determination, the method was investigated by including standards with known molecular weights. A PSS standard with M_w of 17 000 g/mol (supplier's value) were analysed, and we obtained a M_w of 17 200±500 g/mol ($n=6$). A polydisperse PSS was also analysed, and the chromatogram and the calibration curve are given in Fig. 5a together with results obtained for lignosulfonate F-45. The calibration curve of PSS lies below that of the lignosulfonate. This shows that the lignosulfonates have more compact structures than PSS, and that the molecular weight of the lignosulfonates will be underestimated if calculated against PSS standards, in agreement with earlier investigations of similar substances [9]. The two curves are not parallel, and the difference increases with increasing molecular weight. This result suggests that the lignosulfonates are not strictly homologous in terms of asymmetry or conformation. Presumably, the largest molecules ($M_w>10^5$) are relatively more compact and symmetric than the smaller ones.

Fig. 5b includes results obtained for sample F-45 using a TSK G3000 PWXL column and a standard

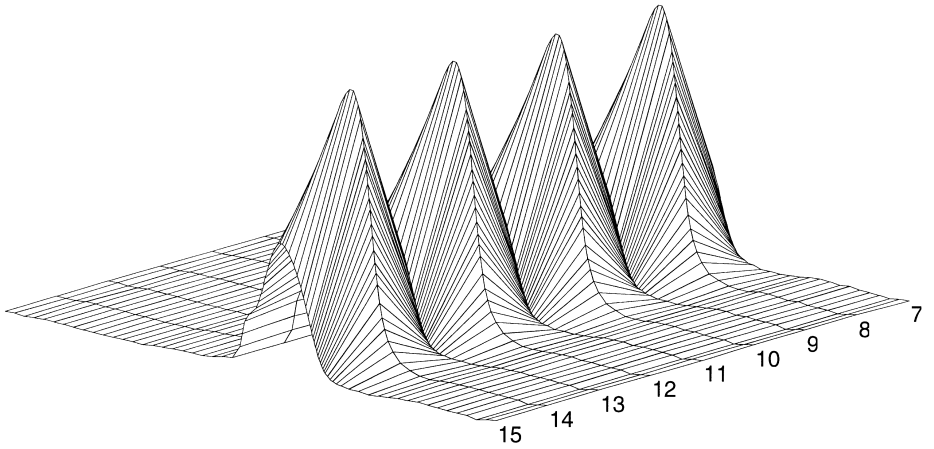

Fig. 4. Light scattering chromatograms with and without interference filters for fraction F-45. Detector 15, 13, 11, 9 and 7 are equipped with interference filters.

aqueous buffer (0.05 *M* Na_2SO_4 containing 0.01 *M* Na_2SO_4) in the absence of DMSO and SDS), but with 20% acetonitrile added. A highly flexible polysaccharide (pullulan) standard with M_w of 16200 was also included. As for PSS the plot of log *M* versus volume for the lignosulfonate lies a factor 2–4 above that of pullulan, again demonstrating the compact structure of lignosulfonates. The column was not able to separate the highest molecular weight molecules, and the more porous TSK columns would be needed to improve the results.

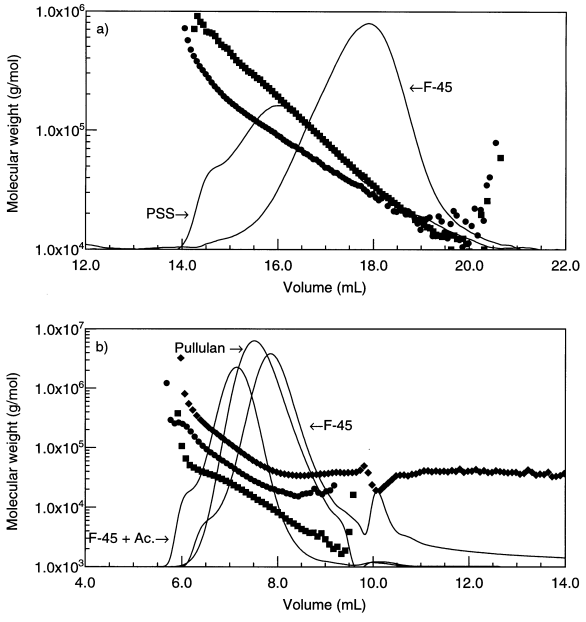

Fig. 5. Calibration plots and elution curves. (a) Fraction F-45 (■) and PSS (●). (b) Fraction F-45 with (●) and without (♦) acetonitrile in the mobile phase, and pullulan (■) with acetonitrile in the mobile phase using TSK-column.

4. General conclusions

We have developed an HPLC-based SEC–MALLS method that can be generally used for determination of the molecular weight and the molecular weight distribution of lignosulfonates. The need for inclusion of DMSO–SDS or acetonitrile in the SEC mobile phase as well as fluorescence filters in the light scattering photometer is clearly demonstrated. Values for d*n*/d*c*, which depend on M_w and the degree of sulfonation, and A_2 (in the SEC solvent) have been determined. The SEC–MALLS method is fast and molecular weight can be calculated over a broad range (5000–400 000 g/mol) in a single experiment. In a forthcoming article we shall investigate the molecular weight distributions of a series of different lignosulfonate samples (different origins/softwood/hardwood) by the presently developed method.

Acknowledgements

The authors wish to thank Research Director Trond Rojahn for valuable and helpful discussions. We gratefully acknowledge technical and economical support from Borregaard LignoTech, Norway.

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