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Note

High-performance size-exclusion chromatography of lignosulphonates

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Lignin is a widely abundant, complex, terrestrial plant polymer which can be removed from plant cell-wall material by a rather large number of chemical means[1]. One very well established method is by sulphonation, which results in the formation of a complex mixture of sulphonated lignins. These water-soluble lignosulphonic acids are widely polydisperse in nature and some have average molecular weights of $> 100\,000$ (ref. 2). It is noteworthy that of the many lignin types available, sulphonated lignins are the most widely used in industrial applications[1].

Since it was first established that lignosulphonates can be fractionated by Sephadex gel chromatography on the basis of molecular size differences[3,4] numerous investigators have used the technique to determine the molecular weight distribution (MWD) profiles of various lignin preparations, *e.g.* lignin sulphonates[5–11], kraft lignins[12–16], milled wood lignins[17–19], organosolv lignins[20], lignin–carbohydrate complexes[21–23], thiolignins[24], etc. The previously reported MWDs of many of these lignin preparations (other than the lignosulphonates) has been questioned, however, because of the discovery that they may be subject to molecular association[16]. The nature of these associations and the methods for suppressing them has been the focus of several recent reports[12,16,20].

High-performance liquid chromatographic (HPLC) methods have, so far, essentially been applied only to the size-separation of relatively low-molecular-weight lignins (number average and weight average molecular weight, $\bar{M}_n$ and $\bar{M}_w$, > 7000) which are soluble in organic solvents, *e.g.* steam exploded wood lignins[25–27], lignin–carbohydrate complexes[28], as well as organosolv[29], dehydrogenative polymerized[30] and milled wood[31] lignins.

The use of HPLC in aqueous media for lignosulphonates, however, has only been the focus of two reports[32,33]. In the latter study, using polystyrene sulphonates of known molecular weights as reference standards, it was reported that a commercial ammonium lignosulphonate (Peritan) had a much lower $\bar{M}_w = 6700$ and $\bar{M}_n = 2600$ than had been expected.

Because of the difficulty in obtaining lignin standards of narrow MWD, many investigations use polystyrene (or polystyrene sulphonates) whose molecular architecture is presumably considerably different from that of lignin. In this paper, we have used lignosulphonates of known $\bar{M}_w$ to demonstrate that a previously described HPLC method for sulphonated lignins[32] fractionates these materials according to molecular-size differences.

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MATERIALS AND METHODS

Instrumentation

The instrumentation used in these experiments was a Waters M-45 solvent delivery system, fitted with a 721 system controller, a 730 data module, a WISP 710 B automatic injection module, and a Model 481 variable-wavelength detector set at 280 nm. Depending upon the molecular size range being studied, chromatographic columns were connected to the WISP in either I-250/I-125 or I-125/I-60 sequences. The mobile phase consisted of filtered (0.8 μm), degassed 50 m*M* citric acid–disodium hydrogen phosphate buffer at pH 3.0. Chromatography was performed on lignosulphonate samples dissolved in the pH 3.0 buffer at a sample concentration of 0.1% (w/v). The mobile phase flow-rate was 1 ml min^{-1}.

Delignification of black spruce

Black spruce chips (5 kg, dry weight) were suspended in a solution of sodium sulphite (25 l, 6.48% total sulphur dioxide, 5.26% free sulphur dioxide) in a closed autoclave. The temperature was then raised to 136°C gradually in 166 min, and held there for an additional 131 min. The maximum pressure recorded was 110 p.s.i.g. The pulping liquor was then removed by filtration to yield, after copious washing, spruce wood pulp (2.34 kg, 46.7% yield, kappa number[1] = 20.8). The spent pulping liquor was used as the source of lignosulphonates in this study.

Preparation of lignosulphonate molecular weight standards

The lignosulphonates were freed from other wood components, and separated into fractions by chromatography on Sephadex G-50 or G-75 by eluting with either 0.25 *M* lithium chloride or water (for desalting) in a manner already described elsewhere[4]. Various fractions were selected and the $\bar{M}_w$ values (Table I), were obtained by a short-column sedimentation technique[2].

RESULTS AND DISCUSSION

It is well-known that during sulphite-promoted delignification of wood, $\bar{M}_w$ of the soluble lignin increases as delignification proceeds[2]. Reported molecular weights ($\bar{M}_w$) of the non-dialysable (> 500) portion range from 7500 to 143 000, representing stages of 25% and 95% delignification, respectively[2].

In this study, black spruce (*Picea mariana*) was almost completely delignified and the resulting crude lignosulphonate mixture used to obtain standards of known $\bar{M}_w$. They were obtained by fractionation of a crude lignosulphonate mixture on a Sephadex gel column, under conditions known to effect separations based upon size differences[4]. The $\bar{M}_w$ values of these fractionated lignosulphonates were then determined by a short-column sedimentation (ultracentrifugation) technique[2] with the results shown in Table I.

The HPLC elution profile of each sample was then recorded, and the curves were then superimposed to give the diagrams shown in Fig. 1, which show that the separation of the lignosulphonate fractions was in the order of decreasing molecular size. The figure also shows that the I-250/I-125 set gave better separation of lignosulphonates with higher $\bar{M}_w$ values, *i.e.* fractions I ($\bar{M}_w$, 120 000) and II ($\bar{M}_w$, 41 000)

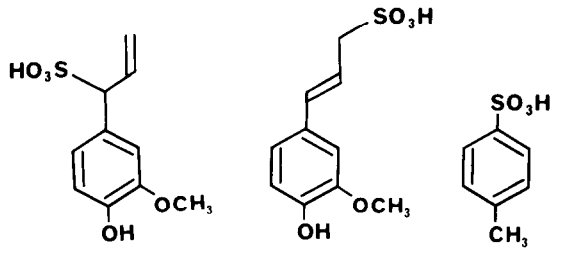
TABLE I

MOLECULAR WEIGHTS OF FRACTIONATED LIGNOSULPHONATES

V_0 = void (exclusion) volume. n.d. = not determined.

Sample	$\bar{M}_w$	*Elution volume (ml)*	
		I-250/I-125	*I-125/I-60*
Blue Dextran 2000 (BD)	2 000 000	12.10	11.41 (V_0)
I	120 000	13.66	11.41 (V_0)
II	41 000	14.77	11.98
III	12 000	16.31	12.90
IV	11 800	16.72	13.20
V	4800	n.d.	14.29
VI	4200	18.45	14.83
VII	3300	19.85	15.77
VIII	244*	22.61	19.74
IX	244*	23.81	21.80
X	156*	n.d.	23.66

* Molecular weight of model compound sulphonates (H form):



VIII IX X

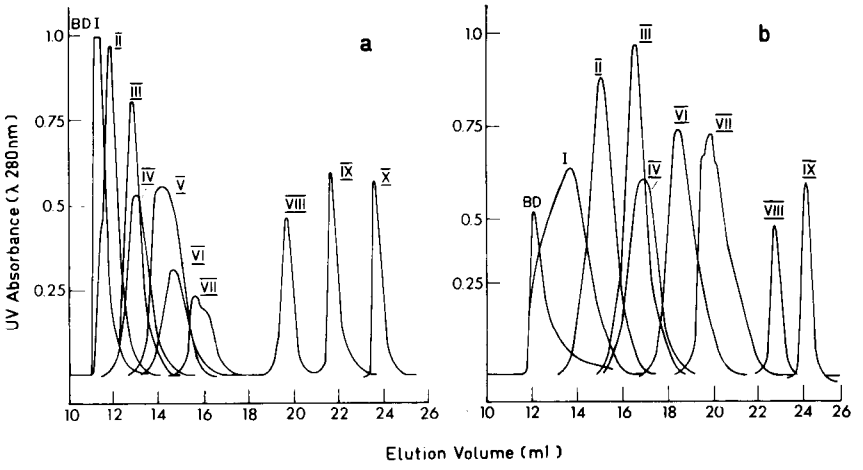


Fig. 1. HPLC elution profiles of fractionated lignosulphonates (I–VII) and sulphonated model compounds (VIII–X). (a) Waters I-125/I-60; (b) Waters I-250/I-125. Eluent: 50 m*M* citric acid–disodium hydrogen phosphate at pH 3.0.

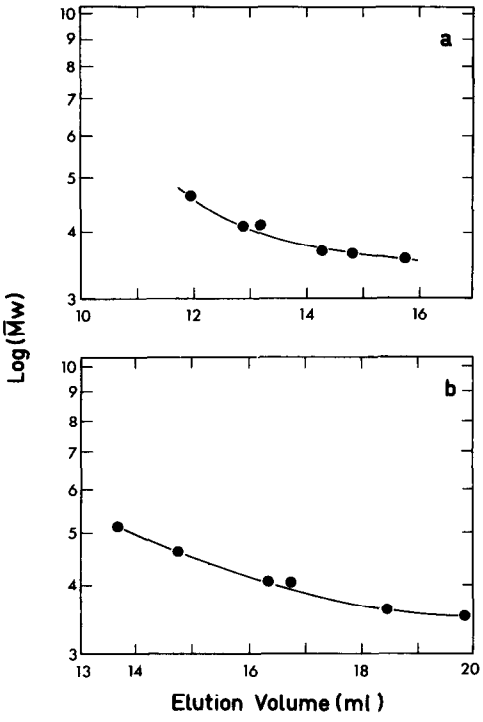


Fig. 2. Calibration curves for lignosulphonates. (a) $\bar{M}_w$ range 41 000–3300 (I-125/I-60). (b) $\bar{M}_w$ range 120 000–3300 (I-250/I-125). Elution details: see Fig. 1.

in Figs. 1a and 1b, respectively. However, the resolution of the lower-molecular-weight components ($\bar{M}_w$, < 4000) was better with the I-125/I-60 column series.

Fig. 1 also shows that the two sulphonated monomers VIII and IX, derived from coniferyl alcohol, are also well-separated. This effect was also observed during Sephadex column chromatography of these compounds, except that the resolution was predictably poorer[32]. While the reason for the separation of these isomers remains unknown, such effects were not observed at higher $\bar{M}_w$ values. Fig. 2 shows the calibration curves obtained for the lignin sulphonate standards ($\bar{M}_w$ range in-

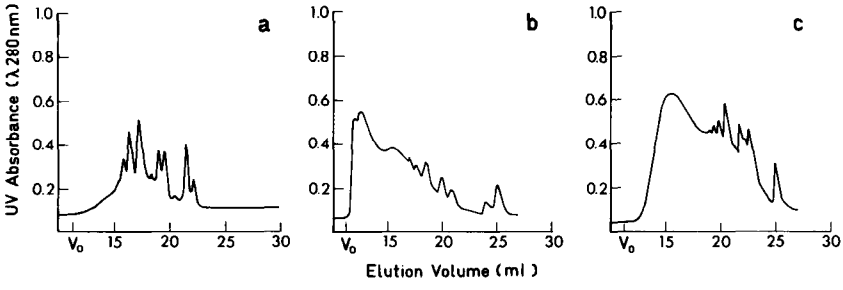


Fig. 3. HPLC elution profiles of soluble sulphonated lignin following 30% (a) and 95% (b,c) lignin removal from black spruce. Columns: (a,b) Waters I-125/I-60 and (c) Waters I-250/I-125. Elution details: see Fig. 1.

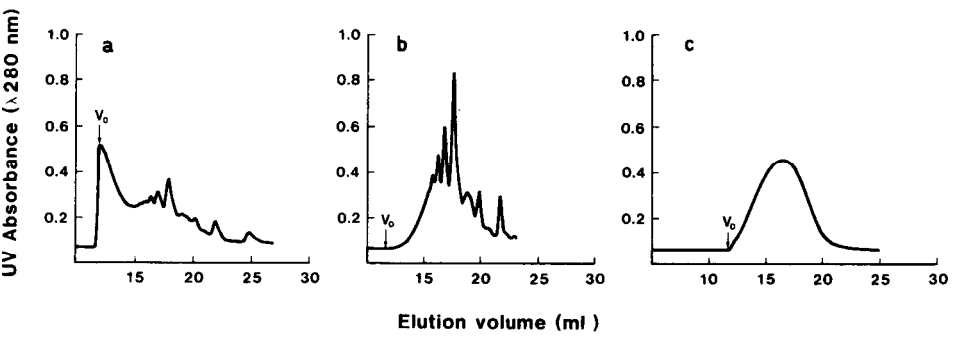


Fig. 4. HPLC elution profiles of a crude lignosulphonate mixture before (a) and after membrane filtration (b,c). (b) Membrane filtrate and (c) membrane retentate. For elution details: see Fig. 3.

cluded 3300–120 000) on I-250/I-125 and I-125/I-60 sets. Again the separations are clearly a function of molecular size differences.

Fig. 3 shows the HPLC elution profiles of soluble sulphonated lignins under conditions effecting partial ($\approx$ 30%, Fig. 3a) and almost total ($\approx$ 95%, Fig. 3b and c) lignin removal. Comparing the elution times in Fig. 3a with those of the lignosulphonate standards (Table I), it indicates that these fairly well-resolved substances have a $\bar{M}_w < 3300$, *i.e.* only monomeric and oligomeric materials with a degree of polymerization of $\approx$ 13–14 have been solubilized. With increased lignin removal (Fig. 3b and c), the more polydisperse materials ($\bar{M}_w > 3300$) are not as well resolved, even with the I-250/I-125 set which has a functional ($\bar{M}_w$) operating range of 0–120 000. A similar lack of resolution was observed during Sephadex column chromatography[32].

This HPLC technique can be used to monitor rapidly the membrane filtration of lignosulphonate mixtures. This can be seen in Fig. 4, which shows the elution profiles of a crude lignosulphonate mixture before and after membrane filtration. It should therefore be obvious that with appropriate filtration procedures and calibration, lignosulphonate samples of known $\bar{M}_w$ can be prepared.

In summary, the advantages of this method are (i) lignosulphonates can be separated by HPLC on the basis of decreasing molecular size. As previously noted, the I-250/I-125 column series is more amenable for chromatography of the more polydisperse materials ($\bar{M}_w$, 3300–120 000) whereas the I-125/I-60 series is more useful for the $\bar{M}_w < 12\,000$; (ii) it is more rapid than conventional Sephadex gel chromatography; (iii) the resolution of the oligomeric components ($\bar{M}_w \leqslant 3300$) is much improved; and (iv) calibration of the columns was carried out with lignosulphonates.

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