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Size-exclusion chromatography of lignin as ion-pair complex

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Abstract

A high-performance ion-pair size-exclusion chromatography method was developed for analysis of underivatised lignin samples using styrene–divinylbenzene gel columns with tetrahydrofuran as solvent. An extraction method using the quaternary amine methyltrioctylammonium chloride allowed quantitative extraction of kraft lignin, lignosulfonic acids and Organosolv lignins from alkaline solution to various organic solvents. Quaternary-amine complexes of lignins formed were analysed by an ion-pair type size-exclusion chromatography in 20 m*M* quaternary amine in tetrahydrofuran. Intermolecular association and adsorption on the matrix was overcome without sample derivatisation. It was possible to analyse various types of lignins and dehydropolymer-lignin model compounds using the same universal analytical system.

Keywords: Ion-pairing reagents; Lignin; Lignosulfonic acids

1. Introduction

Among the natural compounds, lignin is next to cellulose the second most abundant substance in the biosphere. Lignin covers a wide range of polymers constituted of methoxyphenolhydroxypropane units and varies in composition depending on the plant materials. After isolation from wood pulp, lignins may also have different functional groups as a result of the different processing methods. Phenol and methoxy groups can be changed in number and new groups can be introduced. Not naturally occurring in lignins, functional groups such as sulfate or sulfonate can also be introduced into the lignin macromolecule during industrial delignification. Distribution of the molecular mass of lignins can be studied using size-exclusion chromatography (SEC) and introduction of modern high-performance chromatography with pressure stabile gel-supports has provided a rapid and convenient analysis tool for lignin research [1–4]. This method in connection with real-time spectra acquisition by diode array photometer or the Curie-point pyrolysis followed by gas chromatography–mass spectrometry analysis reveals further information about this natural polymer or its synthetic copolymers [5–7]. In another study, crosslinked-lignin in a bead form was also applied as a medium in gel permeation chromatography [8].

However, SEC of lignins displays deficiencies in the form of secondary separation effects due to adsorption on the chromatographic support, of ionic interactions due to the polyelectrolytic nature of lignin, and of intermolecular association. Various chromatographic supports and solvents, e.g., aqueous sodium hydroxide solutions [9], dimethylsulfoxide [4], dioxan–chloroform [10,11], dimethylformamide [12] and tetrahydrofuran (THF) [13], were studied in order to minimise these effects. Inorganic salts such

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as lithium bromide or lithium chloride in dimethylformamide were added to reduce the association between molecules [14]; the addition of zwitterion as betaine or polyethylene glycol was also found to be helpful [9,15]. To overcome the problems of lignin adsorption on the column material, various derivatisation methods such as acetylation [13,16–18], silylation [19] or methylation [20] were applied. Transformation of the lignin macromolecules into less polar products also positively influenced its solubility in less polar solvents, e.g., THF. This low-viscosity solvent is especially common in SEC because it allows good chromatographic resolution and low operating pressure; as well as a high stability of the stationary phase. Derivatisation of lignin minimised the matrix adsorption to some degree but generally did not reduce the associative effects. Overestimation of the molecular mass distribution by these derivatives was demonstrated for lignin model compounds and assumed for lignin samples [21].

Depending on the source of lignins, the functional groups, their counter ions and possible chemical or biological modification of their structure during or after isolation, different chromatographic behaviour can be expected. Most of proposed methods were able to minimise some of the troublesome behaviour but usually displayed other disadvantages. No method so far was universally applicable for even the most common lignin preparations. In this communication, a novel method for applying SEC to lignins is presented which utilises the ion-pairing effect of lignin and quaternary amine (QAM), thus making it possible to overcome most of the problems cited and, in addition, simplifying sample preparation.

2. Material and methods

2.1. Chemicals

Samples of the Organosolv lignins were purchased from Organocell (Munich, Germany). The Organosolv lignin used for the main experiments was isolated from spruce on a technical scale, precipitated with sulfuric acid at pH 4, washed with water and spray dried; other Organosolv lignin samples are listed in the text. Kraft lignin (Indulin AT) was obtained from Westvaco (North Charleston, SC, USA) and lignosulfonic acid (sodium salt) from Roth (Karlsruhe, Germany). Holmen (Karlsruhe, Germany) and Lignotech (Karlsruhe, Germany) generously donated samples of other technical lignosulfonic acids. A QAM, methyltrioctylammonium chloride or methyltridecylammonium chloride, was obtained from Aldrich (Steinheim, Germany) as Aliquat 336. All other chemicals were obtained from Merck (Darmstadt, Germany) and were analytical-reagent quality. Commercial THF containing antioxidant or used eluent was freshly distilled prior to analysis.

2.2. Acetylation of Organosolv and kraft lignin

Dry lignin samples (50 mg) were acetylated in a standard manner using 0.5 ml acetic acid anhydride in 0.5 ml dry pyridine. Samples were incubated at room temperature for 72 h and evaporated in vacuum after addition of 1 ml toluene. Evaporation was repeated with additional 1 ml toluene, dry samples were dissolved in THF and kept frozen.

2.3. Sodium borohydride reduction of kraft lignin

Kraft lignin (Indulin AT) was reduced with sodium borohydride according to the method of Adler et al. [22] as modified by Marton and Adler [23]: a 3 g weight of lignin was dissolved in 180 ml of ethanol–water (1:1) by addition of 50 ml 0.1 *M* sodium hydroxide. A 1.2 g weight of sodium borohydride was added to this solution followed by addition of another 0.1 g of the reagent on each of the subsequent four days. Lignin was isolated after precipitation with ether.

2.4. Standard lignin extraction procedure with QAM

Lignin solution (1–10 mg/ml) was prepared by dissolving a dry lignin sample in 1 *M* sodium hydroxide. A 2 ml volume of this solution and 2 ml of 50 m*M* solution of QAM in ethyl acetate were pipetted to a centrifuge tube with a PTFE lined screw-cap. Lignin was extracted for 30 min using a horizontal shaker and samples were centrifuged for better phase separation. A 1 ml volume of the

organic phase containing lignin was removed, transferred to the new tube and washed with an equal volume of 1% aqueous sodium chloride solution. After centrifugation, an aliquot of 0.5 ml of the ethyl acetate phase was transferred to a sample vial, evaporated in vacuum at room temperature overnight and stored at −20°C. Prior to analysis, the sample was dissolved in 1.25 ml of fresh distilled THF resulting in 20 m*M* final concentration of QAM and 0.4 to 4 mg/ml lignin. A 20 to 50 μl volume of this sample solution was analysed by ion-pair SEC.

2.5. High-performance liquid chromatography system

The high-performance liquid chromatography system was HP 1090M (Hewlett-Packard, Waldbronn, Germany) with an automatic injector, diode array detector and Pascal Work Station with Hewlett-Packard GPC-Software. The column set packed with 6 μm TSK styrene–divinylbenzene polymer type material (TosoHaas, Stuttgart, Germany) consisting of a HXL-L guard column (40×6 mm), one G3000HXL column and one G4000HXL column; both 300×7.8 mm size coupled in order of increasing pore size.

2.6. Ion-pair SEC

Analysis was run in an isocratic mode with a flow-rate of 1 ml/min at a constant temperature of 40°C. The mobile phase was the fresh distilled THF containing 20 m*M* of QAM. The solvent was prepared as a concentrated solution and after filtration through 0.2 μm filter diluted to the required concentration. The system was calibrated with nine polystyrene standards (weight-average molecular mass, M_w, 2 700 000, 470 000, 200 000, 110 000, 34 500, 10 200, 3100, 1050, 450) and biphenyl (M_w 154). Sample volumes of 20–50 μl were generally used. The elution of lignin was detected by absorption at 280 nm with a 4 nm band width; polystyrene standards were detected at 254 nm.

3. Results and discussion

The solubility of lignins in organic solvents differs depending on the isolation procedure, functional groups, molecular mass and also any physical pretreatment. A mixture of solvents is usually much more effective than its single components. In the case of water, alkaline solutions with a pH above 8 and low ionic strength are generally very effective; usually all lignin preparations are readily soluble in strong alkali solutions or in organic solvents mixed with such solutions. All lignin samples used in this study were readily soluble in sodium hydroxide at room temperature. In rare cases, e.g., by some lignins precipitated with strong mineral acids, a gentle heating to 60°C speeded up the solubilisation. Addition of a QAM solution in water immiscible organic solvent and vigorous shaking resulted in immediate formation of a lignin–QAM ion-pair (or complex) and extraction of lignin. Recoveries of kraft lignin, Organosolv lignin and lignosulfonic acid by one step extraction with different organic solvents with 50 m*M* QAM from their solutions (1 mg/ml) in 1 *M* sodium hydroxide are presented in Table 1 and compared to the extraction using pure solvents. In contact with some solvents lignins precipitated between the phases after the formation of QAM complexes or formed a thin third liquid layer between the initial phases. Six organic solvents tested—dichloromethane, toluene, benzene, diethyl ether, *tert.*-butylmethyl ether and ethyl acetate—allowed the extraction of over 92% of the lignosulfonic acid in only one extraction step. Kraft lignin complexes were very readily extracted (>90%) with ethyl acetate, dichloromethane, toluene and the two ethers. Organosolv lignin complexes were recovered to over 96% with all solvents except chloroform, isooctane, butanol and ethylmethylketone. It was possible to apply ethyl acetate, dichloromethane, toluene and the two ethers for the extraction of all three types of lignin. However, ethyl acetate gave the best overall results and, because of low toxicity, low boiling point and moderate price, was the solvent of choice for general application. Remaining lignins may be extracted by second extraction and no fractionation of lignins was detected. After removing an extraction solvent by evaporation in vacuum, lignins extracted with QAM could be easily dissolved in many organic solvents including solvents miscible with water such as dioxan or THF; the QAM–lignin complex was not soluble in water.

To study the dependence of the extraction ef-

Table 1
Extraction of lignins with organic solvents. Percent of total Organosolv lignin, kraft lignin (Indulin AT) and sodium-lignosulfonic acid (LS-Na) extracted from 1 *M* sodium hydroxide (lignin concentration 1 mg/ml) with various organic solvents (1:1, v/v) without (native) and with 50 m*M* QAM in one extraction step.

Extraction solvent	Native lignin			Lignin–QAM complex		
	LS-Na	Indulin AT	Organosolv	LS-Na	Indulin AT	Organosolv
Ethyl acetate	22.7	37.8	62.1[a]	92.3	97.3	99.5
Chloroform	9.9	4.3	0	78.6[a]	76.3[a]	84.4[a]
Dichloromethane	9.2	3.3	1.4[a]	97.1	91.5	96.7
Toluene	2.0	1.6	0	97.3	92.1	97.4
Benzene	4.3	0.5	0	96.5	88.2	97.9
n-Butanol	0	0	0	1.8	2.4	5.4
Isooctane	4.9	3.5	2.6	85.9[b]	85.3[b]	88.6[b]
Diethyl ether	25.1	9.2	4.3	97.5	93.2	96.4
Ethylmethylketone	0	12.0	6.0	32.2	71.0	74.3
tert.-Butylmethyl ether	13.4	1.0	3.0	94.0	94.2	96.9

[a] Part of lignin precipitated between phases
[b] Third layer of lignin complex was formed

ficiency on the concentration of QAM, extraction of lignin was examined in a system consisting of 2 ml 1 *M* sodium hydroxide containing lignin in a concentration 1 mg/ml and 2 ml ethyl acetate with varying QAM concentration. Concentration of QAM of 20 m*M* was sufficient for a practically quantitative extraction of Organosolv lignin and a concentration of at least 15 m*M* QAM was necessary for a constant extraction efficiency of kraft lignin and lignosulfonic acid (Fig. 1). To keep the extraction procedure as universal as possible for all types of lignin, a higher concentration of 50 m*M* QAM in ethyl acetate was chosen as a working concentration. This amount of

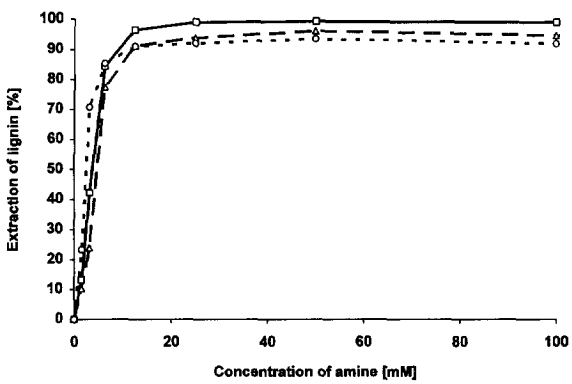

Fig. 1. Lignin (1 mg/ml) extracted from 1 *M* sodium hydroxide solution–ethyl acetate (1:1, v/v) with different concentrations of QAM expressed as percent of total lignin: Organosolv lignin (□—), kraft lignin (△- - -) and lignosulfonic acid (○- - - -).

amine was indeed sufficient for a practically complete extraction of lignin in all cases in which we applied this procedure for extraction of native and biologically or chemically modified lignins.

In contrast, the capacity of QAM to extract lignin from an alkaline solution was also tested. Lignin in varied concentration was extracted in one step from 1 *M* sodium hydroxide solution with an equal volume of 50 m*M* QAM solution in ethyl acetate. The amount of the extracted lignin was presented as a peak area after chromatography in relation to the lignin concentration in the initial alkaline solution (Fig. 2). Lignosulfonic acid was linearly extracted from 1 *M* sodium hydroxide up to the concentration of 15 mg/ml lignin. Extraction of Organosolv lignin, kraft lignin and sodium borohydride reduced kraft lignin was practically linear up to a concentration of about 12 mg/ml. The resulting QAM–lignin solutions in THF were sufficient for even low sensitivity UV detectors and allowed on-line recording of full UV spectra by using a 20 μl injection volume.

After removal of ethyl acetate the QAM–lignin, which was viscous because of an excess of amine, remained stable for months if kept refrigerated or frozen. It was readily soluble in THF and stable in this solution, but to prevent formation of epoxides, samples were dissolved prior to analysis. An attempt was made to elute QAM–lignin complexes from styrene–divinylbenzene polymer type column material as it is the most common packing material for

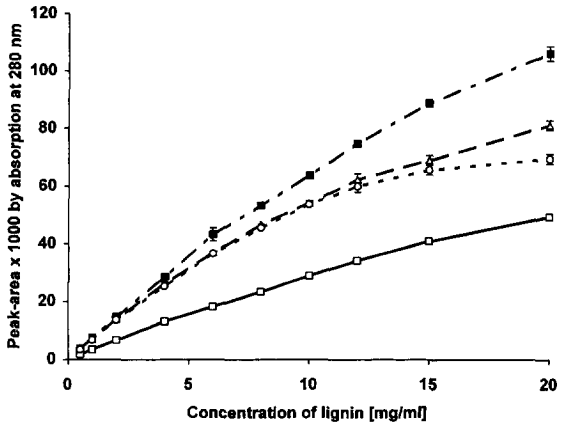

Fig. 2. Recovery of sodium-lignosulfonic acid (□—), kraft lignin (△- - -), sodium borohydride reduced kraft lignin (○- - - -) and Organosolv lignin (■- - -) from their solutions in 1 *M* sodium hydroxide by extraction with 50 m*M* QAM in ethyl acetate. Peak areas were calculated after SEC.

SEC. Dynamic ion-pairs of QAM and lignin were kept soluble by using THF with amine as a solvent. A prepacked column in THF was equilibrated in a solution of 20 m*M* QAM in THF by flushing the column with several column-volumes of the solvent. To prevent a possible corrosion of stainless steal by chloride ions, the column and the high-performance liquid chromatograph were always flushed with a few volumes of pure THF after analysis. QAM in THF and the repeated changing of the solvent did not influence the column resolution if the principal operation conditions of SEC columns were considered. The styrene–divinylbenzene column packing material was not changed in volume by adding QAM to the solvent and showed an equal calibration curve with polystyrene standards as in pure THF (Fig. 3).

The Organosolv lignin is readily soluble in several organic solvents and in mixtures of the same with water but we found that it was not completely dissolved in THF leaving a small amount of brown precipitate after centrifugation. Underivatised kraft lignin (Indulin AT) and lignosulfonic acid sodium salt were only slightly soluble in pure THF and are thus not accessible for SEC in this solvent. An example of separation of Organosolv lignin (native and acetylated) and acetylated kraft lignin using styrene–divinylbenzene polymer matrix and pure THF is given in Fig. 4. Organosolv lignin was

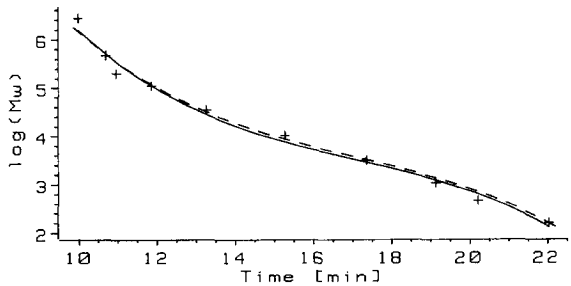

Fig. 3. Calibration curves with nine polystyrene standards (M_w 2 700 000, 470 000, 200 000, 110 000, 34 500, 10 200, 3100, 1050 and 450) and biphenyl (M_w 154) in pure THF (dotted line) and 20 m*M* QAM in THF (solid line) using the TSK column set: flow-rate, 1 ml/min; column temperature, 40°C; detection by absorption at 254 nm (band width 4 nm).

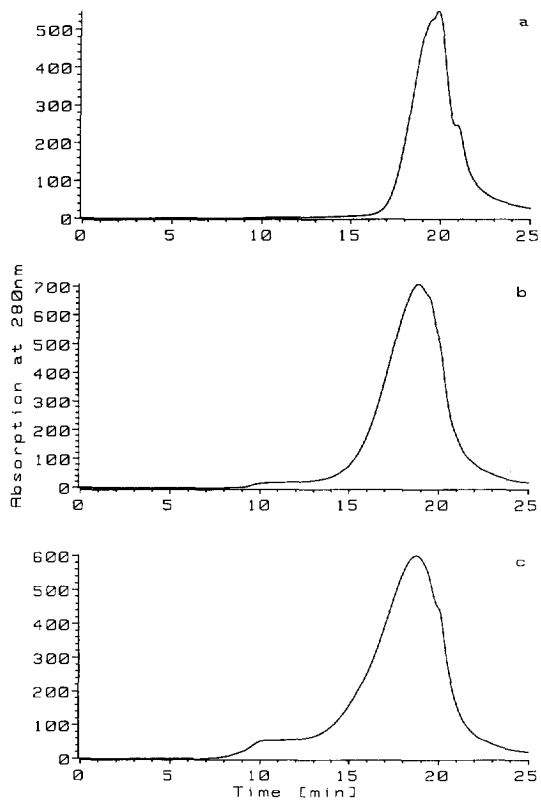

Fig. 4. Size-exclusion chromatograms of Organosolv lignin: native (a) and acetylated (b), and acetylated kraft lignin (c). Apparatus: columns, TSK column set G3000HXL and G4000HXL (300×7.8 mm); solvent, 1 ml/min pure THF; column temperature, 40°C; detection by absorption at 280 nm (band width 4 nm); lignin concentration, 4 mg/ml; injection volume, 20 μl.

Table 2
Molecular mass distribution of lignins in THF. Values calculated from size-exclusion chromatogram using TSK column set G3000HXL and G4000HXL (300×7.8 mm); solvent, 1 ml/min pure THF; temperature, 40°C; detection by absorption at 280 nm (band width 4 nm); lignin concentration, 4 mg/ml; injection volume, 20 μl.

Lignin sample	M_n	M_w	M_w/M_n
Kraft lignin (Indulin AT)—acetylated	1 300	15 200	11.9
Organosolv (spruce, pH 4)	670	1 200	1.8
Organosolv (spruce, pH 4)—acetylated	1 100	6 900	6.4

strongly adsorbed on the column matrix and showed extremely low number-average molecular mass (M_n) and M_w values (Table 2). A significant part of lignin eluted outside the calibration/calculation range as a strongly tailing peak. Acetylation of the sample did not improve the situation even if an excess of acetic anhydride and elevated temperatures were applied. In the acetylated sample a part of lignin eluted in a higher-molecular-mass range but the shape of the peak showed an apparent aggregate-effect. Higher M_n and M_w values were calculated (Table 2) and the polydispersivity increased from 1.8 to 6.4. Kraft lignin could be readily dissolved in THF after acetylation; however, the chromatographic behaviour was very similar to that of the Organosolv sample. The formation of aggregates in a high-molecular-mass range resulted in a bimodal elution pattern and was even more significant in this sample leading to an overestimation of M_n and M_w values (Table 2); peak tailing led to exclusion of a part of the sample from the processing. Bimodal elution as an effect of intermolecular association was very often observed by SEC of lignin and has been the subject of numerous studies [9,24–26].

Transformation of lignin to QAM complexes and analysis using QAM in THF made it possible to overcome the cited problems to a great extent (Fig. 5). The tailing of the lignin samples was minimised and the elution profile was shifted to a higher-molecular-mass range. A bimodal elution pattern was no longer observed and front-tailing in the high-molecular-mass range disappeared. Sodium borohydride reduced lignin with an increased number of hydroxyl groups showed the exact same elution pattern as the original sample with the exception of the low-molecular-mass components removed during

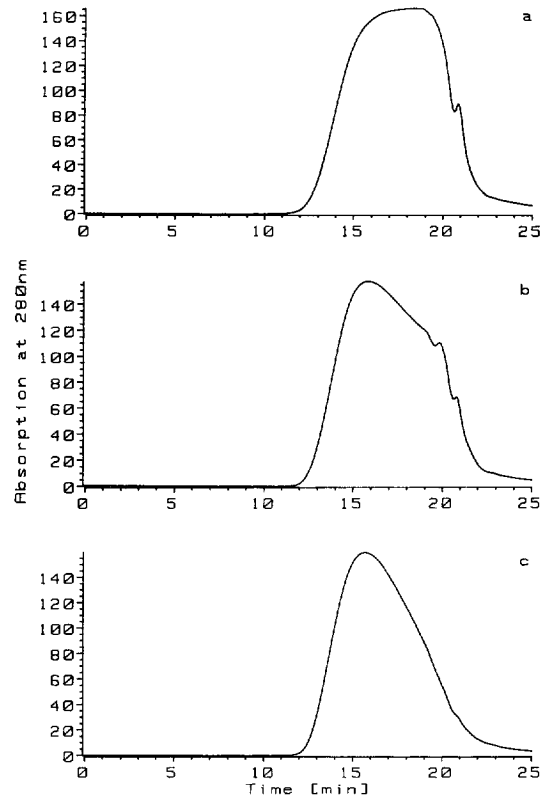

Fig. 5. Ion-pair size-exclusion chromatograms of Organosolv lignin (a), kraft lignin (b), sodium borohydride reduced kraft lignin (c). Apparatus: solvent, 1 ml/min 20 m*M* QAM in THF; other conditions as in Fig. 4.

precipitation of the reduction product with ether. Calculated M_n and M_w values (Table 3) were comparable to values cited in the literature which display large variance with regard to the delignification process and the composition of wood samples.

SEC of lignosulfonic acid requires water compatible column material and was generally performed in aqueous solutions of different pH and salt concentrations (e.g., Refs. [15], [27]). Bimodal elution due to intermolecular association was visible for lignosulfonic acid in aqueous solvent and could not be fully eliminated even by higher salt concentration [3,7,24]. The method presented here was also applied to samples of lignosulfonic acid extracted as QAM complex (Fig. 6 Table 3) making SEC of lignosulfonic acids in organic solvent possible for the first time.

A concentration of a few millimoles of QAM in

Table 3
Molecular weight distribution of industrial lignins. Values calculated from ion-pair size-exclusion chromatograms obtained in 20 m*M* QAM in THF; other conditions as in Table 2.

Lignin sample	Source	M_n	M_w	M_w/M_n
Kraft lignin (Indulin AT)	Westvaco	1 700	6 100	3.5
Kraft lignin (Indulin AT)—borohydride reduced		2 000	7 400	3.3
Organosolv (spruce, pH 4)	Organocell	1 500	5 400	3.6
Organosolv (spruce, pH 9)	Organocell	1 800	7 100	4.0
Organosolv (spruce, excess methanol, pH 4)	Organocell	1 500	5 300	3.6
Organosolv (spruce, excess methanol, pH 9)	Organocell	1 700	6 600	3.8
Organosolv (poplar, pH 4)	Organocell	1 200	4 100	3.4
Sodium-lignosulfonic acid	Roth	3 900	30 400	7.8
Sodium-lignosulfonic acid	Holmen	3 500	32 300	9.1
Ammonium-lignosulfonic acid	Holmen	4 300	53 400	12.2
Wafex	Holmen	4 000	36 200	9.0
Wafex CAM	Holmen	4 200	55 000	13.2
Wafex SR	Holmen	3 900	35 800	9.2
Diwatex 30FKP	Lignotech	3 400	22 000	6.4
Diwatex 40P	Lignotech	4 100	29 600	7.2
Curan 100	Lignotech	2 200	11 100	5.0
Wanin-Mg	Holmen	3 800	31 800	8.3
Zewa EF	Lignotech	3 900	33 700	8.7
Zewakol-MgN	Lignotech	2 800	19 800	7.0
Zewakol-Ca	Lignotech	3 600	35 200	9.8

eluent was necessary to prevent the precipitation of free lignin and irreversible adsorption on the column matrix. Although the lignin–amine complexes were rather stable in organic solvents, they are thought to be in dynamic equilibrium with the dissolved QAM and are thus comparable to ion-pairs formed by conventional liquid chromatography with tertiary or quaternary amines, or sulfonated alkanes. The chromatographic behaviour of Organosolv lignin, kraft lignin and sodium-lignosulfonic acid was studied using different concentrations of QAM in THF. The calculated molecular mass expressed here as M_n and M_w values was only slightly influenced by increasing the amine concentration over 20 m*M* (Fig. 7). This effect was much less pronounced than by addition of inorganic additives as in previously reported methods. The effect of QAM on lignin is comparable to the interaction of inorganic ions and not to chemically bonded unpolar groups as introduced by derivatisation. M_n and M_w values moved towards each other and the resulting polydispersivity was thus also minimised by increasing QAM concentration. To keep the signal-to-noise ratio as small as possible for the analysis of low lignin concentrations and for on-line recording of absorption spectra, the lowest possible amine concentration in eluent was preferred; 20 m*M* of QAM was found to be a good compromise.

The method described was applied to different commercially available lignins (for further examples see Fig. 8 and Table 3) and lignins modified by enzymatic or fungal treatment. The dominance of high-molecular-mass compounds in lignin precipitated with carbon dioxide (pH 9) in comparison to the same lignin precipitated with sulfuric acid (pH 4) was well demonstrated. The chromatogram obtained from an acid precipitated sample of poplar Organosolv lignin revealed a characteristic low-molecular-mass distribution. It was also possible to analyse samples of dehydropolymers obtained from guaiacol or coniferyl alcohol with peroxidases using the aforementioned method (data not shown). In the case of low solubility of such polymers in pure aqueous alkaline solution alone, samples were well dissolved and extracted by dispersion of a dry sample with equal volumes of 1 *M* sodium hydroxide and 50 m*M* QAM in ethyl acetate simultaneously.

Application of QAM in mobile phase and trans-

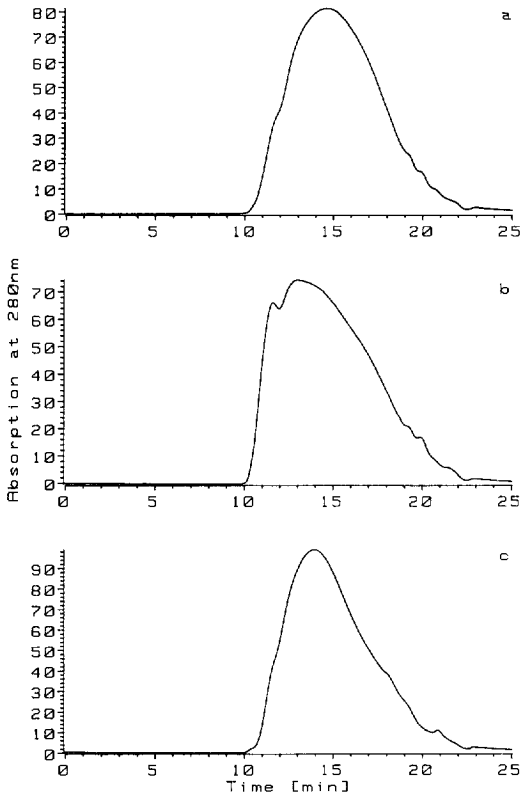

Fig. 6. Ion-pair size-exclusion chromatograms of industrial lignosulfonic acid: sodium-lignosulfonic acid from Roth (a), ammonium-lignosulfonic acid from Holmen (b), Diwatex 40P from Lignotech (c). Apparatus: solvent, 1 ml/min 20 m*M* QAM in THF; other chromatographic conditions as in Fig. 4.

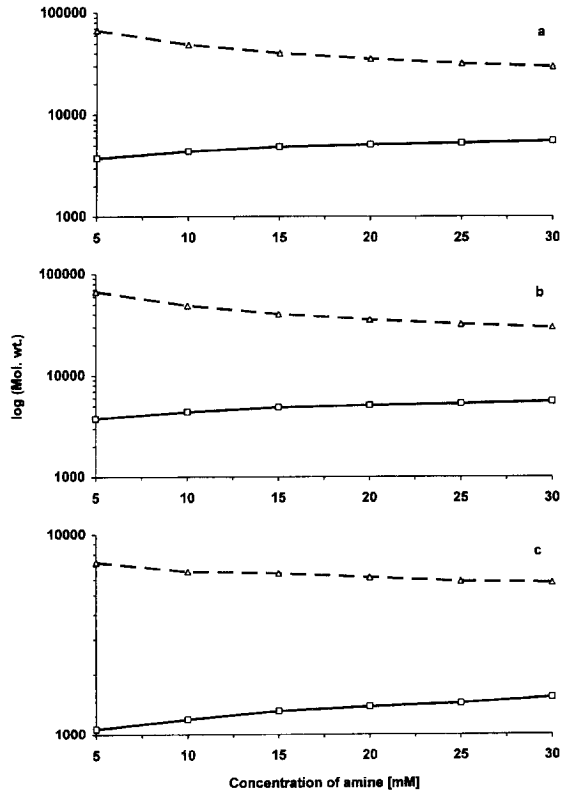

Fig. 7. M_n (□—) and M_w (△- - -) values of sodium-lignosulfonic acid (a), kraft lignin (b) and Organosolv lignin (c) in eluent with different concentrations of tridecylmethylammonium chloride (QAM) in THF; other chromatographic conditions as in Fig. 4.

formation of lignin to the QAM complex made it possible to overcome the common problems of lignin analysis. Additionally, the lignin was highly purified from water soluble materials such as sugars, cellulose, polysaccharides or other fungi products which remained unextracted. Single washing of the organic phase with a 1% solution of sodium chloride was necessary for removing traces of sodium hydroxide from the organic phase. Salt solution allowed better phase separation and, in single cases, prevented precipitation of lignin between the phases. Omission of this step caused a negative peak and thus signal disturbance in the low-molecular-mass range. Other column matrices, e.g., silica-based Zorbax PSM Type S materials, could also be used; however, the physical stability of these types of materials stands in contrast to their inherent tendency to irreversibly adsorb lignin, thereby shortening the column life time. The calibration of the column with polystyrene standards was not the optimal but was the only available choice; it shared the problems common to all methods. In certain cases calibration with fractionated lignin samples [28] helped in overcoming these problems. In connection with a proper detector, such as the real-time differential viscometer, the reliability of molecular mass determination would be significantly and universally increased [29]. A problem which in our opinion remains unsolved is the applicability of common GPC-software and calculation methods in the characterisation of such heterogeneous polymers as lignins. Commonly used M_n, M_w, or polydispersivity values are useful in describing homogenous synthetic polymers eluted as one, more or less symmetrical peak but they cannot be applied to lignins without reservation. Application

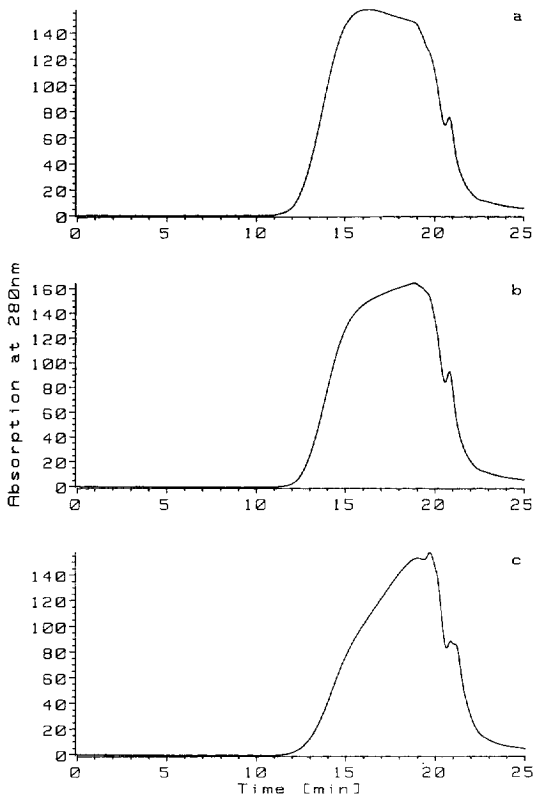

Fig. 8. Ion-pair size-exclusion chromatograms of Organosolv lignins in 20 m*M* QAM in THF: lignin obtained with excess methanol from spruce, precipitated at pH 4 with sulfuric acid (a), precipitated at pH 9 with carbon dioxide (b), poplar lignin precipitated at pH 4 (c); other chromatographic conditions as in Fig. 4.

of these values for characterisation of lignin samples can lead to erroneous results and does not reflect the real mass distribution. A division of the calibrated mass range into several molecular mass ranges and the calculation of mass-percent in these ranges should accompany the common GPC calculations. As long as no reliable calibration standards are used or no universal detection of molecular mass is possible, comparisons of samples with a standard sample would be preferable to any absolute calculations.

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