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HIGH-PERFORMANCE SIZE-EXCLUSION CHROMATOGRAPHY OF LIGNIN AND ITS DERIVATIVES

J. PELLINEN* and M. SALKINOJA-SALONEN

University of Helsinki, Department of General Microbiology, Mannerheimintie 172, 00280 Helsinki (Finland)

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SUMMARY

High-performance size-exclusion chromatography of different lignins was studied using styrene–divinylbenzene copolymer gel columns. Acetylated and silylated model compounds were compared with underivatized samples to see the effect of possible adsorption or intermolecular association on the chromatography. The results showed that these phenomena did not cause any major interference in the chromatographic system described here. Derivatization increased the average molecular weights of lignins by more than was calculated from the hydroxyl content. We believe that the derivatization changed the hydrodynamic volume of the molecules, so the calibration compounds should also be changed. Alternatively, this might reflect some kind of association between the non-polar groups in the lignin molecules. For this reason the average molecular weights of lignin cannot be calculated by subtracting the mass of derivative groups from the average molecular weight of derivatized samples. Polystyrene calibration was found unsuitable in the analysis of lignin derivatives. The lignin model compound calibration seemed to be promising.

INTRODUCTION

High-performance size-exclusion chromatography (HPSEC) provides a rapid and convenient way to obtain information on the molecular weight distribution (MWD) of polymers. SEC of lignin-related material can suffer from artifacts due to adsorption on the gel, ionic interactions, or intermolecular association[1–5]. The extent of adsorption depends on the combination of the gel and solvent[1,3]. The adsorption can be hydrophilic or hydrophobic and leads to an underestimation of the molecular weight (MW). Ionic interactions are due to the polyelectrolytic nature of dissociated lignin in alkaline water solutions[6].

The question of the effect of intermolecular association in SEC of lignins has been discussed by Sarkanen *et al.*[7]. It has been reported that the MW of an association complex can be as much as three orders of magnitude larger than the true MW[1,7]. It has been suggested that hydrogen bonding or hydrophobic interactions between lignin molecules can lead to the formation of association complexes[8,9] and

bimodal elution curves in SEC[1]. Lindberg[8] studied thiolignins by infrared spectroscopy and found that hydrogen bonding of hydroxyl groups caused formation of association complexes. It has also been suggested that long-range van der Waals forces may lead to association[10]. Association can be avoided by using a strongly polar mobile phase, such as dimethyl sulphoxide (DMSO) or dimethylformamide (DMF), which form hydrogen bonds with the sample molecules and solvate them completely[1,4,11,12]. Tetrahydrofuran (THF) can break the association dimers that are often formed in the solutions of carboxylic acids, without leading to extensive solvation[5]. Another possibility for diminishing the adsorption and association caused by hydroxyl groups is to remove them by derivatization. Acetylated[12-15], methylated[16], and silylated[17] lignins have been used for this purpose, and also to increase the solubility of lignin. However, Ekman and Lindberg[16] did not find any significant differences in the elution profiles of methylated and underivatized lignins. The nature and even the existence of association complexes of non-polar lignin derivatives have remained very unclear.

In a previous study we examined SEC of lignins in aqueous mobile phases[18]. In the present study we evaluated the effects of adsorption and molecular association on the average MWs of lignins with a HPSEC system consisting of styrene–divinylbenzene copolymer gel and THF as the mobile phase. The free hydroxyl groups in different lignins and lignin model compounds were acetylated or silylated to eliminate hydrogen bonding between the target molecules. The MWs of derivatized and underivatized substances were then compared. Our results indicate that neither adsorption nor hydrogen bonding caused major artifacts in the chromatographic system described here.

EXPERIMENTAL

Model compounds

The structures of the model compounds used are presented in Fig. 1 and their names in Table I. Compound I was obtained from EGA-Chemie (Steinheim/Albuch, F.R.G.), II from BDH (Poole, U.K.) and III from Fluka (Buchs, Switzerland). Compounds IV–VIII were gifts from Dr. K. Lundquist, Chalmers Technical University, Gothenburg, Sweden. The acetylated form of compound IX was a gift from Dr. G. Brunow, University of Helsinki, Helsinki, Finland. The underivatized form of IX was prepared by alkaline hydrolysis. Compound X was synthesized by Dr. G. Brunow et al. as described in the literature[19] using 2-guaiacoxy-3-(3-methoxy-4-hydroxyphenyl)-1,3-propanediol as the starting material. The purity and identity of the product were verified by high-performance liquid chromatography (HPLC), mass spectrometry and nuclear magnetic resonance spectrometry.

Fractionated polystyrenes (PS) were used to calibrate the columns (Pressure Chemicals, Pittsburgh, PA, U.S.A.). The MWs of the PSs as given by the manufacturer were 50 000, 17 500, 10 300, 4000, 2200, and 800. The void (V_0) and total permeation (V_t) volume were determined with PS 50 000 and acetone (MW 58, E. Merck, Darmstadt, F.R.G.), respectively. The void volume was also checked with a high MW lignin sample.

Distribution coefficients (K_d) were used instead of retention volumes in Fig. 2: $K_d = (V_r - V_0)/(V_t - V_0)$, where V_r is the retention volume of model compound[5].
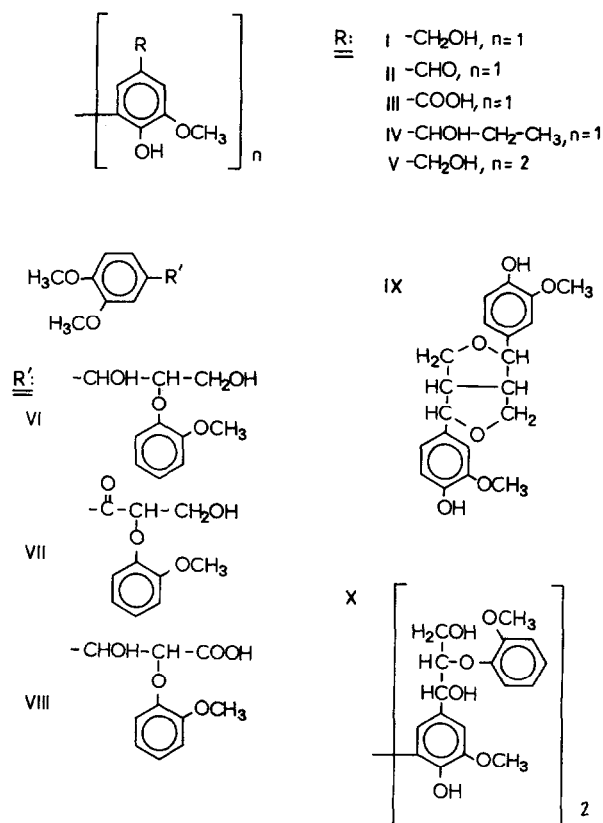
Fig. 1. Lignin model compounds used in this study.

TABLE I

NAMES AND MOLECULAR WEIGHTS OF MODEL COMPOUNDS

No.	Name	MW
I	3-Methoxy-4-hydroxybenzyl alcohol	154
II	3-Methoxy-4-hydroxybenzaldehyde	152
III	3-Methoxy-4-hydroxybenzoic acid	168
IV	1-(3-Methoxy-4-hydroxyphenyl)propanol	182
V	2,2'-Dihydroxy-3,3'-dimethoxy-5,5'-dihydroxymethylbiphenyl	234
VI	1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol	334
VII	1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)-3-hydroxypropanone	332
VIII	2-(2-Methoxyphenoxy)-3-(3,4-dimethoxyphenyl)propionic acid	348
IX	2,6-Bis(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane	358
X	1,1'-(4,4'-Dihydroxy-5,5'-dimethoxy-3,3'-biphenylene)-bis[2-(2-methoxyphenoxy)-1,3-propanediol]	638

Explosion wood lignin (EXWL) from birch was prepared using the system described in the literature[20]. The pressure was 27 kg/cm², for 4 min, and the temperature 220°C. The product was extracted with water (48 h) and dioxane (48 h), and finally the dioxane extract was evaporated to dryness. The result of an elemental analysis was: 62.76% C and 6.50% H; the total hydroxyl content was found to be 7.63%[21].

Explosion wood lignin from poplar was a gift from Dr. S. Malhotra, Xerox Research Center, Canada (Iotech, Ontario, Canada). It was prepared at 40 kg/cm², at 250°C with a steaming time of 1 min, and purified by extraction with water and ethanol–water. Elementar analysis gave the values of 57.80% C and 5.44% H; the total hydroxyl content of a similar preparation was determined[22] to be 9.82%.

Softwood kraft lignin (pine), Indulin AT-R, (63.37% C, 5.57% H, 11.14% OH) was a gift from Westvaco, North Charleston, SC, U.S.A. The elemental analyses
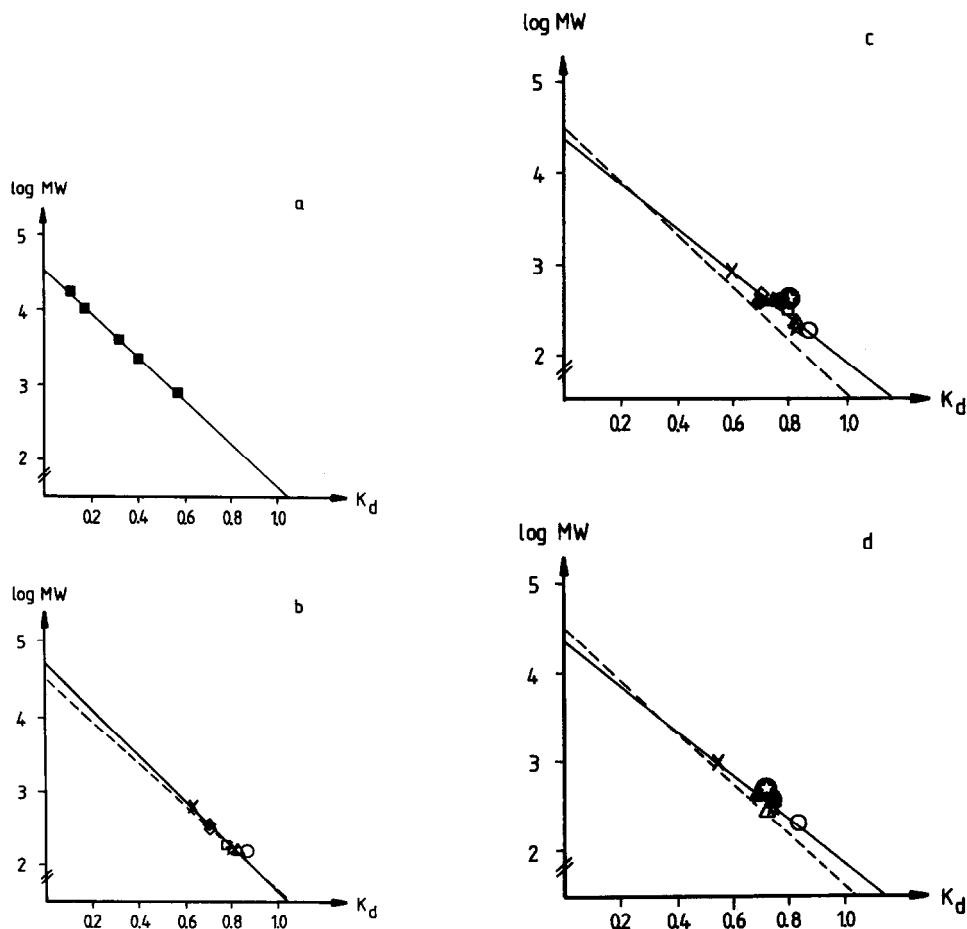

Fig. 2. Elution of model compounds. (a) Polystyrene standards; (b) underivatized; (c) acetylated; (d) silylated samples. The solid line is the regression line of the model compounds, the broken line in b–d is the polystyrene calibration line. Symbols: △ = I, ○ = II, ☆ = III, □ = IV, ◇ = V, ▲ = VI, ● = VII, ◉ = VIII, ◆ = IX, × = X, ■ = PS.

were carried out at the Federal Research Center for Forestry and Forest products, Hamburg, F.R.G., by Mr. U. Engel using a Carlo Erba Elemental Analyser Model 1104. All the samples were stored −18°C.

Derivatization

Acetylation. The sample (1–15 mg) was dissolved in 0.5 ml of freshly distilled, dry pyridine (J. T. Baker, Deventer, The Netherlands); an equal amount of distilled acetic anhydride (E. Merck) was added, and the mixture was allowed to stand for 24 h at 21°C. Then 0.25 ml of methanol (E. Merck) was added to the ice-cooled reaction mixture, and it was evaporated to dryness with a Speed Vac Concentrator (Savant Instruments, Hicksville, NY, U.S.A.). Next, 0.5 ml of freshly distilled toluene (E. Merck) was added and the sample was again evaporated to dryness. The procedure with toluene was repeated twice to remove all the traces of solvents. Finally, the sample was dissolved in THF (HPLC grade, Rathburn, Walkerburn, Peeblesshire, U.K.), filtered with a 0.47-μm PTFE filter (Gelman Sciences, Ann Arbor, MI, U.S.A.) and injected into the chromatograph.

Silylation. The sample (1–15 mg) was dissolved in 400 μl of THF (dry, redistilled); 40 μl of redistilled pyridine and 400 μl of N,O-bis(trifluorotrimethylsilyl)-acetamide (E. Merck) were added and the bottles (1.5 ml) were purged with nitrogen. The reaction time was 24 h at 21°C. The sample was evaporated to dryness with the vacuum concentrator, dissolved in THF, filtered, and injected. Reversed-phase HPLC and gas chromatography were used to check the derivatization.

Apparatus

The chromatographic system has been described earlier[18]. Ultrastyragel 1000 Å and 500 Å styrene–divinylbenzene copolymer columns (30 × 0.8 cm I.D., Waters Assoc., Milford, MA, U.S.A.) were used in series, with THF as mobile phase at a flow-rate of 1.00 ml/min. The curves were digitized by hand by measuring the height of the signal from the baseline at 0.33-ml intervals. These slices were then integrated with a microcomputer; the areas of the slices were calculated as a percentage of the total area and plotted against the logarithm of the corresponding MWs. With this presentation, the changes in the sample concentration, injection volume or column set did not affect the distribution curve; furthermore, the calibration was also taken into account. Alternatively, the weight fraction of the slices could be calculated as shown in ref. 5.

RESULTS

Calibration with model compounds

PS standards, derivatized as well as underivatized model compounds, were used for the calibration studies. The plots of log MW *vs.* the distribution coefficient (K_d) are shown in Fig. 2. For comparison the regression line of the PS standards (Fig. 2a) is also drawn in Fig. 2b–d (broken line). The exclusion limit of the system was found to be *ca.* 30 000 g/mol. The injected concentration of the model compounds was less than 10 μM, to prevent association[9,23].

The results shown in Fig. 2 indicate that no significant adsorption of the model compounds on the gel occurred, as all the K_d values were less than 1.0. Also severe

artifacts due to the formation of association complexes were absent, since the K_d values of underivatized model compounds agreed well with those of the PS standards, and no multiple peaks occurred (Fig. 2). By comparing Fig. 2a and b with Fig. 2c and d it can be seen that the derivatized model compounds eluted later than the PSs and underivatized compounds. Reversed-phase adsorption is not likely to explain this delay because the model compounds eluted in the order of decreasing MW and not in the order of decreasing polarity (increasing number of non-polar groups in the molecule).

It is possible that the elution of the underivatized model compounds was affected by strong solvation which might then lead to low K_d values[24]. The solvation of the derivatized model compounds may be less intense in THF, and the molecules may therefore have eluted later than expected on the basis of the elution of the unmodified parent compounds. There was no sign of intermolecular association in the chromatography of the model compounds.

Lignin samples

The studies with lignin model compounds showed that their elution volume depended on the MW as well as on the derivatization. It follows that the calibration should be done using the same derivatization procedure for both the samples and the calibration compounds. Since no lignin-like model compounds were available for the calibration of the high MW range (MW > 10^3), PS calibration was used in the calculation of the average MWs (Table II) and in the transformation of the chromatograms to distribution curves (Figs. 3–5). For comparison, the average MWs were also calculated with the calibration lines obtained by using corresponding model compound derivatives (Table II).

The model calibration usually gave somewhat higher values of the number average MW ($\bar{M}_n$) and lower values of the weight average MW ($\bar{M}_w$) than the PS calibration (Table II). Both calibrations gave similar low values of $\bar{M}_n$ for the underivatized samples, indicating that solvation was probably not as intense as in the

TABLE II

AVERAGE MOLECULAR WEIGHTS AND POLYDISPERSITIES OF THE SAMPLES

Sample*		PS calibration			Model compound calibration		
		$\bar{M}_n$	$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_n$	$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$
Birch	u	970	4880	5.03	1180	6640	5.64
(EXWL)	a	1440	9210	6.38	2000	7370	3.68
	s	2160	12110	5.60	2530	9350	3.70
Poplar	u	770	2280	2.97	930	3000	3.23
(EXWL)	a	850	3300	3.89	1300	3250	2.50
	s	1340	5200	3.88	1690	4600	2.72
Pine	u	460	2330	5.07	540	3090	5.70
(kraft)	a	1010	7590	7.54	1520	6240	4.09
	s	1120	7480	6.69	1480	6150	4.17

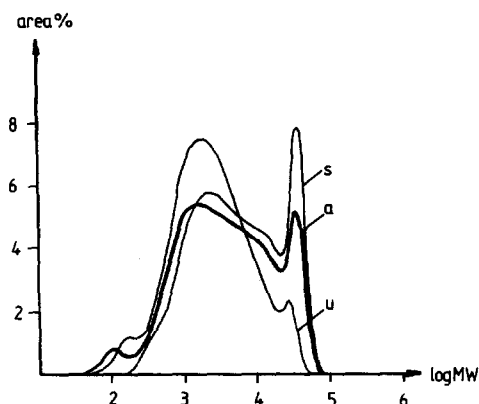* u = underivatized; a = acetylated; s = silylated.

Fig. 3. MW distribution of birch EXWL: u = underivatized; a = acetylated; and s = silylated sample.
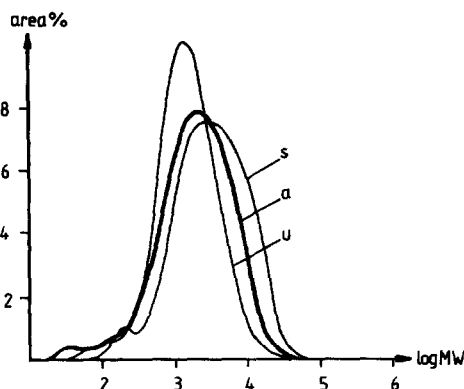
Fig. 4. MW distribution of poplar EXWL; u, a, and s as in Fig. 3.

case of the model compounds. As can be seen from Figs. 3–5, derivatization of lignin noticeably increased the amount of the material of MW greater than 10^4. The theoretical increase in the MW caused by derivatization was calculated from the hydroxyl content (see materials and methods) and average MWs of the underivatized samples, based on the assumption that the reactions were complete (Table III). This method gives only a rough estimate for the effect of derivatization because the decrease in the polydispersity and the amount of carboxyl groups were not taken into account. From Tabel III it can be seen that acetylation and silylation usually increased the observed average MWs by more than was calculated from the hydroxyl content. The increase in $\bar{M}_n$ was 2–4 fold when the PS calibration was used (excluding the value of the acetylated poplar EXWL), and 2–7.5 fold when the model compound calibration was used (Table III). The corresponding increases in $\bar{M}_w$ were 2–9 fold (PS calibration), and 1.5–5 fold (model compound calibration, excluding the values of acetylated birch and poplar EXWL). It follows that the average MWs would be too high if they were calculated by subtracting the mass of the derivative groups from the average MWs of the derivatized samples.
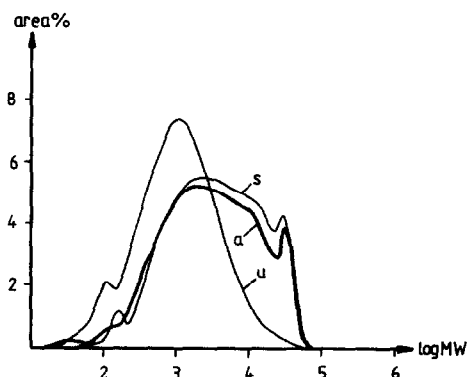

Fig. 5. MW distribution of kraft lignin; u, a, and s as in Fig. 3.

TABLE III

CALCULATED AND OBSERVED INCREASE OF THE AVERAGE MOLECULAR WEIGHTS

ΔM_{calc} was calculated from the hydroxyl content of the sample, assuming that all the hydroxyl groups had reacted; ΔM_{PS} values were calculated from Table II using polystyrene calibration, and ΔM_{der} values from Table II using model compound calibration.

Sample*		ΔM_{ncalc}	ΔM_{nPS}	ΔM_{nder}	ΔM_{wcalc}	ΔM_{wPS}	ΔM_{wder}
Birch	a	180	470	820	920	4330	730
(EXWL)	s	320	1470	1350	1600	7240	2710
Poplar	a	190	80	370	550	1030	250
(EXWL)	s	320	570	760	960	2920	1600
Pine	a	130	550	980	610	5270	3150
(Kraft)	s	220	660	940	1110	5150	3060

* a = increase of MW in acetylation; s = increase of MW in silylation compared with underivatized sample.

The polydisperisty was smaller when the model compound calibration was used. It was interesting to find that the polydispersity seemed to decrease in the derivatizaton when model compound calibration was used but to increase when PS calibration was used (Table II).

DISCUSSION

The underivatized model compounds neither adsorbed the gel nor underwent intermolecular association (Fig. 2b). Therefore we have no reason to believe that the polymeric lignin would associate. The model compounds were so chosen that they contained different structures and functional groups typical for lignin (Fig. 1). Furthermore, the chromatograms showed little tailing, which often reveals adsorption. The possibility of adsorption cannot, however, be overlooked since the apparent $\bar{M}_n$ value of the underivatized kraft lignin was so low.

When the plots of the model compounds are compared with the line for PSs in Fig. 2. It can be seen that the calibration line obtained with derivatized model compounds differs noticeably from that of the PSs and should therefore give more reliable MWs for the derivatized lignin samples. No final conclusions can, however, be drawn since there were no calibration points in the high MW range. Polystyrenes could be used in the calibration of underivatized lignins because the PS line was close to that obtained with the model compounds (Fig. 2a and b). The use of PSs is advantageous making comparisons with other systems possible.

The apparent MWs of lignins increased in the derivatization more than theoretically calculated (Table III). Derivatization may have changed the conformation of lignin, expanding the molecules. Alternatively, some kind of lipophilic association would explain the increase in the MWs. Such association was, however, not observed with the model compounds. The peak at the exclusion volume (Figs. 3 and 5) indicated that the pores of the gel of the columns used were too small to fractionate the largest molecules, and the calculated MWs are thus somewhat too low. The average

MWs from both calibrations were similar for underivatized samples (Table II), and those of the poplar EXWL were close to the values reported in the literature. Glasser et al.[22] reported values of $\bar{M}_n = 600$ (vapour pressure osmometry), $\bar{M}_n = 900$ (SEC), and $\bar{M}_w = 3000$ (SEC, $\bar{M}_w/\bar{M}_n = 3.33$) for a similar exploded wood lignin preparation from poplar. EXWL of aspen has been studied by Marchessault et al.[25] ($\bar{M}_w = 1700-1900$, $\bar{M}_w/\bar{M}_n = 2.4-2.7$) and Himmel et al.[23] ($\bar{M}_n = 925$, $\bar{M}_w = 1980$, $\bar{M}_w/\bar{M}_n = 2.1$).

The observed number average MW of kraft lignin was much lower (540) than earlier reported. For softwood kraft lignins, $\bar{M}_n$ values between 700 and 1700, depending on the procedure used (colligative methods), have been given in the literature[9,12,22,26]. In a recent study values of $\bar{M}_n = 1300$ and $\bar{M}_w = 4300$ (SEC) were reported for softwood kraft lignin[22]. As high values as $\bar{M}_n = 4800$ and $\bar{M}_w = 15600$ ($\bar{M}_w/\bar{M}_n = 3.24$) have been obtained for spruce kraft lignin using SEC[27]. The chromatograms of kraft lignin obtained in this study (Fig. 5), however, resemble those published dpreviously[1,17,28].

The polydispersities of lignins calculated using model compound calibration were similar to those calculated from the PS calibration and decreased in the derivatization (Table II). This, together with the fact that the number average MW of the underivatized kraft lignin was so low, indicates that there may have been some adsorption of the the lignins on the gel, which was eliminated by the derivatization. The lower slopes of the calibration lines obtained with the model compounds also lead to a lower polydispersity. The polydispersity of the birch EXWL was larger (3.68–5.64) than that of the EXWL poplar (2.50–3.23), which indicates that it was less degraded and more heterogenous. Reported polydispersities of lignins have usually been less than 3.0[13,14,23,25,26].

CONCLUSIONS

Adsorption onto the gel or intermolecular association due to hydrogen bonding through hydroxyl groups did not cause any major problems in the chromatographic system described here.

The use of acetylated or silylated lignins was complicated by conformational changes in the molecules in the derivatization and the lack of high MW compounds for the calibration. PS standards can be used to calibrate the columns if underivatized lignins are to be analysed, but this calibration seemed to lead to too large polydispersities of derivatized lignins.

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