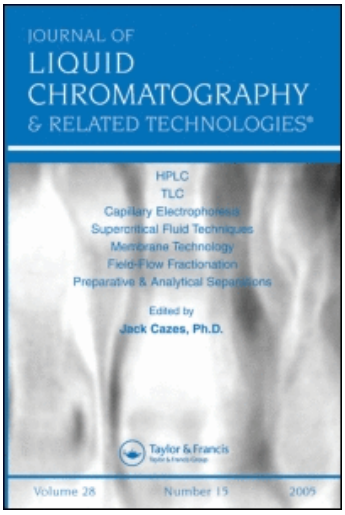

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SIZE EXCLUSION CHROMATOGRAPHY OF LIGNINS USING LITHIUM CHLORIDE/N,N-DIMETHYLACETAMIDE AS MOBILE PHASE. I. DISSOLVED AND RESIDUAL BIRCH KRAFT LIGNINS

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ABSTRACT

The molecular weight distributions (MWDs) of dissolved and residual lignins from different delignification phases during flow-through kraft cooking of birch have been studied. The fundamental characteristics of the isolated lignin samples were evaluated and the lignins were characterised by size exclusion chromatography using lithium chloride/N,N-dimethylacetamide (LiCl/DMAc) not used for isolated lignin samples before. The MWDs of underivatized and acetylated samples were compared with the MWDs of acetylated samples obtained by using tetrahydrofuran (THF) as mobile phase in a similar chromatographic system.

The results indicate that removal of pulp lignin during the initial phase of kraft cooking of birch is restricted partly because of the large size of the lignin polymer. However, at the end of the cook no such restrictions remain. The apparently larger molecular size obtained with the LiCl/DMAc-system compared to the THF-system, may be due to interactions between the polystyrene standards and column matrix in combination with a more extensive conformation of the lignin polymer and/or a higher degree of swelling of the polystyrene-divinylbenzene matrix. Although differently accentuated, the residual lignin samples show a bimodal MWD in both solvents. Possible explanations for this phenomenon may be due to structural heterogeneity in the residual lignin, different molecular conformation due to the polarity of the solvent and/or different degrees of associations between lignin fragments in the two solvents.

INTRODUCTION

Lignin is an amorphous biopolymer and second to cellulose, the most abundant polymer in plants.[1] The common feature of lignins is its heterogeneous polyphenolic alkyl-aryl ether structure. The chemical composition varies with species and morphological origin.[2] In hardwood lignins, referred to as syringyl-guaiacyl lignins, the most frequent monomers are dimethoxyphenolhydroxypropane (syringyl) and methoxyphenolhydroxy-propane (guaiacyl) in roughly equal proportions.[1] The units are mainly linked together by aryl ether linkages either to the hydroxyalkane side chain or to another aromatic unit. Since it is not possible to remove lignin without concomitant modification, the characteristics of a lignin sample also depend on the applied isolation method.[1]

In addition to the above, the chemical composition of lignin samples isolated from process liquors or wood pulps also depend on the process employed for pulp production. The most common delignification process in Sweden is kraft pulping, where hydrogen sulphide and hydroxide ions are the active chemicals. During kraft processing of wood, lignin is partially degraded and dissolved into the black liquor. However, the cook is stopped before the delignification is complete to minimize the coincident degradation of carbohydrate polymers, which cause a loss in pulp strength and yield.

In order to obtain a strong pulp fibre with a low amount of residual lignin, research is continuously undertaken to elucidate the reactions of lignin during pulping. The delignification rate of birch wood during kraft pulping depends on the chemical structure of the lignin, leading to a faster removal of lignin in regions with a higher proportion of syringyl lignin.[3] The dissolution of lignin

also depends on the size of the lignin fragment, since it has to diffuse through the fibre pore.[4] In this context, characterisation of lignin and the carbohydrate polymers with respect to molecular weight distribution (MWD) is of great interest.

Size-exclusion chromatography (SEC) is commonly used to obtain information on the MWD of polymers.[5] In organic SEC, the most common packing material is porous polystyrene-divinylbenzene particles. The separation is based on the physical principle that solutes of different size have different abilities to diffuse in and out of the pores. Sample components with a large size will elute earlier compared with smaller components, which are able to diffuse into the pores to a greater extent. For a given packing material all sample molecules above a critical size are excluded from the pores and will coelute in the same volume, V_o. Below a critical molecular size, corresponding to the total permeation volume, V_p, it is not possible to separate molecules of different size. The selective permeation volume, V_s, which is available for separation is defined as:

$$V_s = V_p - V_o$$

In order to increase the separation range, SEC columns of different pore sizes are often combined. When differential refraction detectors are used, as in this study, the molecular weight distribution is commonly calculated by using narrow standards of known molecular weight.

Under non-ideal SEC conditions, enthalpic interactions can occur which will contribute to the observed retention time.[6] To obtain a separation solely based on differences in molecular size, the conditions should be chosen so that associations between sample molecules and sample-columns interactions are eliminated. Since the solvent used to dissolve the sample also is used as the mobile phase, it should be compatible with the packing material of the column.

Underivatized lignins have been characterised by size exclusion chromatography using aqueous sodium hydroxide[7] dioxane/chloroform,[8] dimethylsulfoxide and dimethylformamide.[9] Although neat dimethylsulfoxide and dimethylformamide dissolves most types of lignin samples readily, association occurs. By adding lithium salts to dimethylformamide, it is possible to dissociate underivatized[9] and acetylated lignin samples.[10] Aqueous sodium hydroxide has also been used to compare the MWD of underivatized lignin and low-molecular weight carbohydrate polymers.[11] One advantage of using an alkaline eluent is that the dissociation of the phenol groups prevents lignin association in dilute solutions.[12] It is also necessary to use fresh alkaline solutions since carbohydrates and lignin are structurally modified upon storage.[11] Recently, size-exclusion chromatography of underivatized lignins as ion-pair complexes[13] has been reported. MWDs of the underivatized lignins

were obtained by addition of a quaternary amine to THF. To increase the lignin solubility and overcome hydrophilic interactions, lignin is frequently derivatized prior to chromatography. THF has been used to characterise acetylated,[14,15] methylated,[16] and silylated[17] lignin samples.

This paper presents a new method to characterise underivatized and acetylated lignins by size-exclusion chromatography, using lithium chloride/N,N-dimethylacetamide (LiCl/DMAc). In contrast to other lignin solvents, LiCl/DMAc is also suitable for dissolution and characterisation of underivatized hardwood kraft pulps by SEC.[18,19] Dissolved and residual lignin from various delignification phases have not earlier been isolated from flow-through kraft cooks of birch. Sample characteristics and changes in MWD during delignification are discussed and the MWDs obtained by using LiCl/DMAc are compared with those obtained with a similar chromatographic system using THF.

EXPERIMENTAL

All chemicals were of analytical grade. Acetone, 1,4-dioxane, hydrochloric acid, lithium chloride, *n*-pentane, sulphuric acid, and tetrahydrofuran were purchased from Merck (Darmstadt, Germany) whereas N,N-dimethylacetamide was purchased from Sigma-Aldrich (Gillingham, UK).

Cooking Conditions and Sample Preparations

Before cooking, dry birch chips were impregnated with deionized water for one hour to facilitate accessibility of the cooking liquor. White liquor, with a composition of hydrogen sulphide and sodium hydroxide to simulate conventional kraft cooking conditions, was continuously added. The initial temperature was 65ºC, raised by 1ºC/min to 165ºC. After the cook, the pulps were washed thoroughly and characterised with respect to viscosity and lignin content.

To obtain samples of dissolved lignins, black liquor was continuously collected in six consecutive fractions during 210 minutes of the cooking time, using a flow rate of 75 mL/min. Dissolved lignins were isolated by acid precipitation of the black liquor fractions essentially according to Gellerstedt *et al*.[20] Fresh black liquors were kept under nitrogen and lignin was precipitated by adjusting the pH to 4.5 by careful addition of 2 M sulphuric acid. The acidified samples were placed in a freezer overnight to ease coagulation of lignin. The following day, the precipitates were separated by centrifugation at 10,700 g for 15 minutes, washed twice by chilled deionized water and then freeze-dried.[21] The acid precipitates were extracted by *n*-pentane to remove elemental sulphur

and extractives. After freeze-drying, the precipitates were extracted with dioxane:deionized water (9:1) to separate lignin from co-precipitated carbohydrates.

Residual lignin was isolated from pulps originating from three different kraft cooks interrupted after 90, 150, and 210 minutes, respectively. In these cooks, the flow rate was kept constant at 50 mL/min. Since the 90 minute-cook was stopped before the wood chips were completely defibrated, this pulp was milled to 4 mm granulates prior to further treatment. All pulp samples were extracted by acetone to remove extractives. The residual lignin was obtained by acid dioxane extraction at 88°C following the method of Gellerstedt *et al*.[22]

Characterization of Wood and Pulp

The viscosity of pulps dissolved in cupriethylenediamine was determined according to ISO 5351/1 (1981). The kappa number was determined according to ISO 302 (1981). Acid insoluble lignin (Klason lignin) was gravimetrically determined after hydrolysis by 82% sulphuric acid at room temperature for two hours, followed by dilution to 3% and autoclave treatment at 120ºC for an additional half hour. Viscosity, kappa number, and Klason lignin were determined at the Swedish Pulp and Paper Research Institute (STFI).

Characterization and Acetylation of Lignin Samples

Mikro Kemi AB, Uppsala, Sweden, carried out determinations of elemental composition and ash content. Methoxyl analysis was performed by Analytische Laboratorien, Elbach, Germany. Acetylation and phenol group determinations of the lignin samples were carried out according to the procedure described by Månsson[23] at STFI.

Size Exclusion Chromatography Using 0.5% LiCl/N,N-dimethylacetamide

50 µg of acetylated or underivatized lignin samples were characterised by a SEC system consisting of an autoinjector AS-4000A (Merck-Hitachi), a L-6200A pump (Merck-Hitachi) and a refractive index detector, RI-71 (Shodex). Characterisations were performed at 35ºC, on three columns connected in series, HR 5E, HR1, and HR0.5 (Waters) using a flow rate of 1 mL/min. The columns were calibrated with polystyrene (PS) standards, in the range 2930 D to 2.56 MD (Polymer Laboratories Ltd., UK). Cubic correlation was used and the degree of correlation between PS and the time scale was $r^2 = 0.9929$. Calculations were carried out with PL Caliber (Polymer Laboratories Ltd., UK).

Size Exclusion Chromatography Using Tetrahydrofuran

40 µg of acetylated lignin was characterised at room temperature. The SEC system consisted of a Rheodyne 7125 injector (Rheodyne), a Waters 510 pump, a set of three columns connected in series, 10^4Å, 500Å, 100Å, (ultrastyragel, Waters) and a flow rate of 1 mL/min was used. The solutes were detected by a Waters 410 refractive index detector (Waters). Polystyrene standards in the range 580 D to 350 kD (Polymer Laboratories Ltd., UK) were used to calibrate the columns. Cubic correlation was used and the degree of correlation between PS and the time scale was $r^2 = 0.9996$. Data acquisition and calculations were carried out with Baseline (Waters).

RESULTS AND DISCUSSION

Characteristics of Pulps and Lignin Samples

In Figure 1 an overview of the wood, pulp, and lignin samples used in this study is shown. Pulping conditions are, besides the isolation procedure, the governing characteristics of the hardwood lignin samples. In order to minimize post-modification of dissolved lignin and re-precipitation of dissolved material that may occur in batch cooking, all cooks were performed in a laboratory flow-through reactor. Kraft cooks are divided into three phases according to the rate of delignification.[24] The lignin samples in this study, represents lignin fragments dissolved in the black liquor and residual lignin remaining in the pulp at different phases of delignification. During the initial phase a small amount of lignin is dissolved. The transition point between initial and bulk delignification for the cooks in this study is assumed to occur during the 60-90 minute interval. During the bulk delignification phase, roughly corresponding to samples collected between 60 and 180 minutes, the main portion of lignin is removed. The pulp and black liquor samples obtained after 180 minutes corresponds to the residual delignification phase, when the delignification rate is lower.

The pulp yield, pulp viscosity, and lignin content of wood and pulps are shown in Table 1. Based on Klason lignin, about 70% of the lignin dissolved during the cook were recovered by acid precipitation. The remaining lignin released consists of low molecular weight hydrophilic fragments, which were not possible to isolate using the applied method.

It is not possible to accurately calculate the efficiency of the isolation method or the recovery of residual lignin samples because of difficulties to determine the lignin content in pulp. Klason lignin is considered to be the best

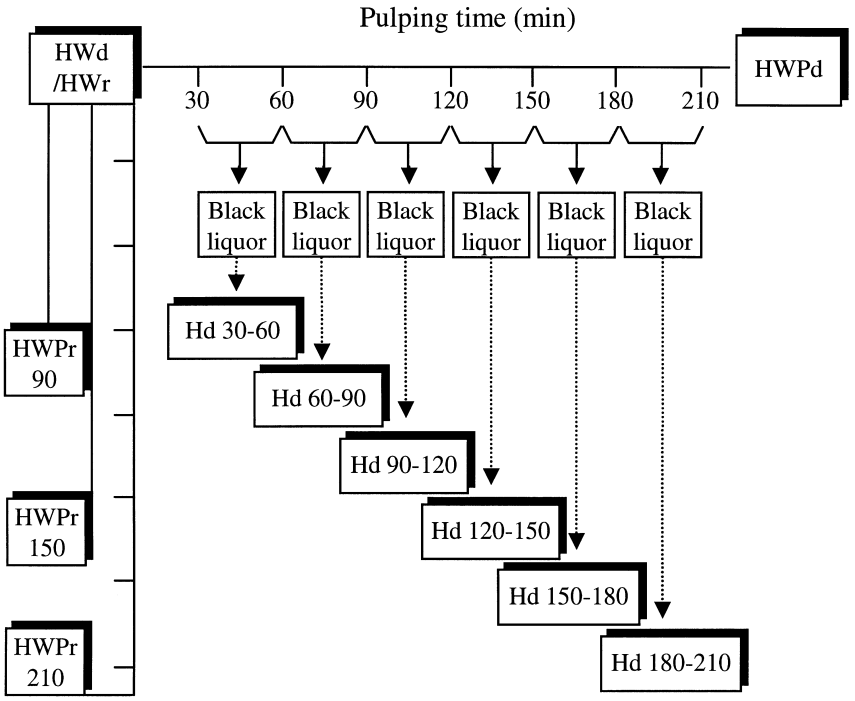

Figure 1. An overview of wood, pulps, and black liquor obtained from flow-through kraft cooking of birch wood. HWd = hardwood chips used in the cook from which dissolved lignin samples were obtained and HWPd = the pulp belonging to it. Hd = hardwood dissolved lignin. The time range refers to the collection time of black liquor from which the sample was isolated. HWr = hardwood chips used to obtain pulps designated HWPr, from different delignification phases. HWPr 90, 150, 210 corresponds to pulps in the beginning and end of the bulk delignification and to the residual delignification phase. The residual lignin samples isolated from these pulps are designated Hr 90, Hr 150, and Hr 210, respectively.

measure of lignin but since it is a gravimetric determination it is inaccurate for pulps of low lignin content. Kappa number is used to estimate the amount of lignin in pulp, but is rather a measure of all structures oxidised by potassium permanganate, i.e. does not exclusively reflect the amount of lignin. Unsaturated structures, such as hexeneuronic acid in hemicellulose, contribute substantially to the kappa number of hardwood kraft pulps.[25] In order to enable comparison of our results with other published results concerning the isolation of residual lignin, kappa numbers of pulps before and after acid dioxane extractions are shown in Table 1.

Table 1

Pulp Yield, Viscosity, and Lignin Content of Pulps Used for Isolation of Dissolved and Residual Lignin

Sample[1]	Pulp Yield (% of Wood)	Viscosity (mL/g)	Klason Lignin (%)	Kappa No. After Cooking	Kappa No. After Extract'n[2]
HWd	---	---	19.7	---	---
HWPd 210	44.5	1290	<0.1	9.2	---
HWr	---	---	18.3	---	---
HWPr 90	64.6	---	13.9	84	27
HWPr 150	48.8	1380	0.5	13	2.9
HWPr 210	46.7	1210	0.1	9.2	2.7

[1] Designation according to Figure 1.
[2] Acid dioxane extraction.

In general, it is difficult to isolate large amounts of residual lignin from pulp without concomitant modification of the lignin. Acid dioxane extraction of pulp at elevated temperature as used in this study is an effective method to obtain residual lignin from kraft pulps. The method has earlier been applied to obtain residual lignins from different parts of a kraft cook of spruce,[26] but for hardwood kraft pulps it has only been used to isolate residual lignin from birch pulp corresponding to HWPr 210.[22] The drawback of the isolation method is that the acid conditions used may modify the structure of the residual lignin samples to some extent. During stronger acidic conditions than those prevailing during acid dioxane extraction, both degradation and condensation of the lignin structure occurs. By comparing acid dioxane extraction of spruce using batch and flow-through reactors, respectively, Jiang *et al.*[26] concluded that the structure of the residual lignin is not altered significantly during this type of isolation procedure. In acidolysis experiments, birch lignins have been reported to have more acid labile ether linkages than spruce.[27] Using oxidative degradation of the phenolic moiety, no evidence for extensive condensation of residual lignin during acid dioxane extraction of birch pulp has been found.[22] Consequently, in spite of the milder conditions used during acid dioxane extraction compared with acidolysis, a certain degradation of the residual lignin obtained in this study cannot be ruled out.

Two important functional groups in kraft lignins are methoxyl and phenol groups. The molecular formula of each sample was calculated from elemental and methoxyl group determinations according to Zakis.[28] Molecular formulas normalised to C_{100} and concentration of phenol groups are shown in Table 2.

Table 2

Normalised Molecular Formula and Concentration of Phenol Groups of Lignin Samples

Sample[1]	Molecular Formula	Phenol Groups mmol/g
Hd 30-60	$C_{100}H_{110}N_{0.7}O_{31.3}S_{12.1}(OCH_3)_{7.30}$	1.7
Hd 60-90	$C_{100}H_{100}N_{0.4}O_{30.1}S_{11.5}(OCH_3)_{10.9}$	2.2
Hd 90-120	$C_{100}H_{9125.9}S_{4.3}(OCH_3)_{15.8}$	2.3
Hd 120-150	$C_{100}H_{92}O_{29.4}S_{3.5}(OCH_3)_{13.8}$	2.6
Hd 150-180	$C_{100}H_{90}O_{25.9}S_{3.6}(OCH_3)_{13.9}$	2.8
Hd 180-210	$C_{100}H_{90}O_{32.4}S_{4.1}(OCH_3)_{13.2}$	2.5
Hr 90	$C_{100}H_{97}O_{29.2}S_{0.47}(OCH_3)_{17.}$	0.86
Hr 150	$C_{100}H_{94}N_{0.3}O_{28.5}S_{1.3}(OCH_3)_{11.9}$	2.3
Hr 210	$C_{100}H_{98}N_{0.6}O_{26.7}S_{1.5}(OCH_3)_{10.2}$	2.5

[1] Designation according to Figure 1.

The high methoxyl content of the samples is due to the syringyl type of lignin in hardwood, and indicates a reasonably high purity with regard to lignin. The methoxyl content of the dissolved lignin is low in the beginning of the cook and reaches a maximum in the middle of the cook. Thereafter, the amount of methoxyl groups slightly decreases and levels out. The methoxyl content of the lignin dissolved early in the cook is slightly lower in the present study compared with batch cooking[29] but except for this difference the relative amounts are approximately the same. The high content of methoxyl groups in Hr 90 indicates that the residual lignin isolated from cooks interrupted in the beginning of the bulk delignification phase mainly consists of syringyl lignin and also that it is possible to isolate lignin of high purity by acid dioxane extraction. The decrease in methoxyl content of the residual lignin during pulping may be explained by demethoxylation of the aromatic ring and also that the syringyl-lignin is more easily de-lignified in comparison to the guaiacyl-moieties of the lignin structure.[3] The increase of phenol groups in the lignin samples during the cook, shown in Table 2, is mainly caused by cleavage of α- and β-aryl ether linkages.[30] The cleavage of ether linkages in phenolic lignin units starts already during the initial phase, while non-phenolic aryl ether bonds require higher temperatures and hence contribute to the formation of new phenolic groups in the lignin during the bulk phase. In the residual delignification phase, cleavage of carbon-carbon bonds contributes to lignin degradation without a concomitant creation of new phenol groups. In accordance with this, the concentration of

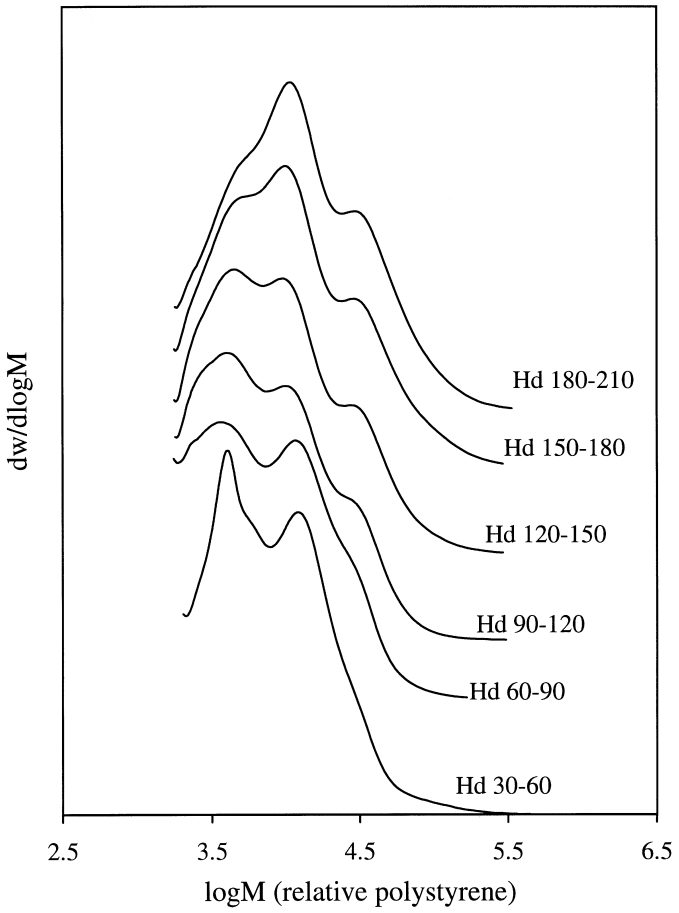

Figure 2. Molecular weight distributions of underivatized dissolved lignin samples isolated from black liquor collected during a kraft cook of birch. Samples are designated as in Figure 1. The samples were chromatographed on a series of three HR-columns using 0.5% LiCl/DMAc as mobile phase.

phenol groups is decreasing in the lignin dissolved during this latter phase. At the end of the cook, the concentration of phenol groups in the residual lignin is equal to that of dissolved lignin. Although to a minor extent, cleavage of methyl-aryl ether bonds in syringyl and guaiacyl structures also contributes to the formation of phenol groups (catechol structures) throughout kraft cooking of birch.[29] The phenol concentrations of the dissolved lignin samples achieved in this study are slightly higher in all delignification phases compared to the phenol

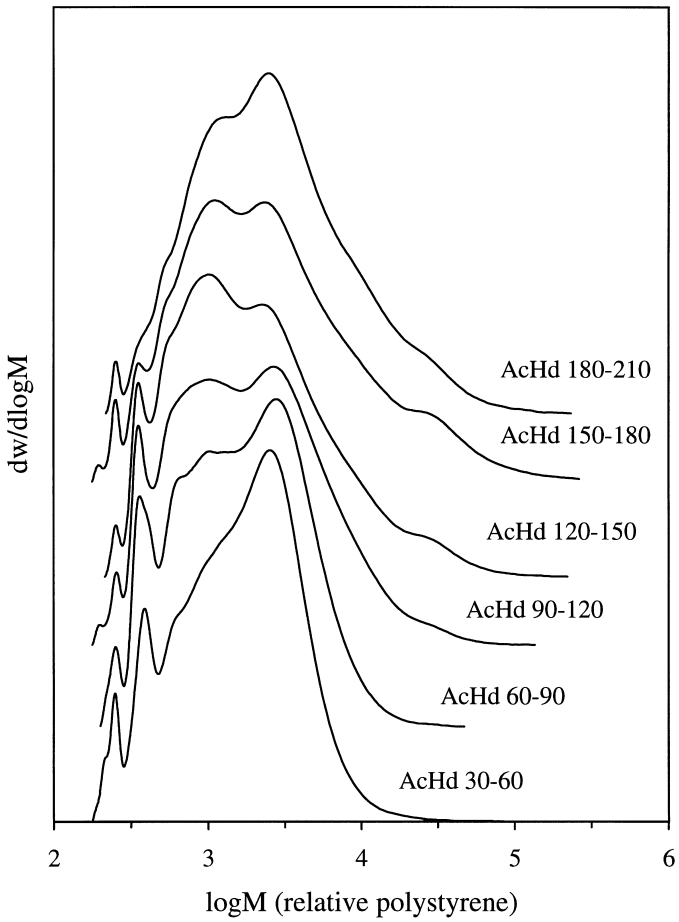

Figure 3. Molecular weight distributions of acetylated dissolved lignin samples isolated from black liquor collected during a kraft cook of birch. Samples designated as in Figure 1. The samples were chromatographed on a series of three ultrastyragel columns using THF as mobile phase.

concentration of lignin samples isolated from a batch kraft cook of birch.[29] A probable explanation for this discrepancy is the use of different analytical methods, the aminolysis method applied in this study is more accurate compared with estimating the amount of phenol groups from potassium permanganate oxidation of lignin samples as used in the study by Gellerstedt *et al.*[29] The elemental composition shown in Table 2 also includes nitrogen, which presumably originates from proteins in the pulp fibre. As commonly seen in lignin samples from kraft cooking, the samples also contain sulphur. The

elemental composition and the change herein of the lignin samples Hd 90-120 - Hd 180-210 is in accordance with results obtained for dissolved lignin from interrupted batch cooks of birch.[29] The elemental composition of the residual lignin at the end of the cook (Hr 210) is quite similar to those obtained from batch cooking of birch.[22]

Size Exclusion Chromatography of Lignin Samples

All lignin samples were easily dissolved in 0.5% LiCl/DMAc. In contrast, it was not possible to dissolve all of the underivatized lignin samples in tetrahydrofuran (THF). To compare the MWDs obtained by LiCl/DMAc, acetylated lignin samples were characterised by a similar chromatographic set-up but using THF as solvent. Ac-prefix is used to designate acetylated samples throughout this report. In both SEC-systems, the molecular weight of the dissolved lignins is continuously increased whereas the molecular weight of the residual lignins is continuously decreased during the cook.

In Figures 2 and 3 the MWDs of the dissolved lignin samples using LiCl/DMAc and THF, respectively are shown. The shapes of the distributions are essentially the same in both systems. In the MWD of the lignin dissolved early in the cook, corresponding to the initial phase and the beginning of the bulk delignification phase, two maximums can be discerned. During the cause of delignification, a third maximum appears and becomes more accentuated at the high molecular end of the distribution. However, except for a small distribution around 30,000 (log 4.5) which is also seen with the LiCl/DMAc-system, the positions of the maximums are located at lower molecular weight ranges when using THF. The increase in molecular weight of the dissolved lignin during delignification of birch wood pulp has also been observed for dissolved lignins isolated from batch[31] and flow-through[14] kraft cooks of pine. In these cases the lignins were silylated and acetylated, respectively, and THF was used as eluent.

The change in molecular weight distribution of residual lignin during kraft cooking of birch has not been studied earlier. In Figures 4 and 5 the MWDs of the residual lignin samples using LiCl/DMAc and THF, respectively, are shown. The samples correspond to the lignin remaining in the pulps in the beginning (Hr 90) and at the end of the bulk (Hr 150) and in the residual delignification phase (Hr 210). Irrespective of which solvent is used, the width of the distributions of the residual lignin samples decreases during delignification i.e. with decreasing molecular weight. All of the distributions are bimodal when using the LiCl/DMAc-system (Figure 4).

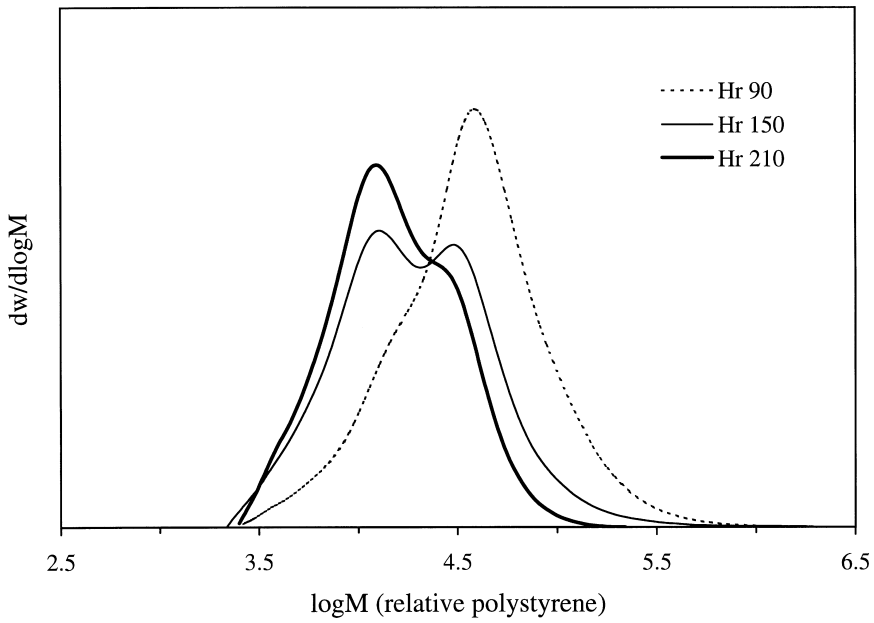

Figure 4. Molecular weight distributions of underivatized residual lignin samples isolated from kraft birch pulps of different degrees of delignification. Conditions are as in Figure 2 and samples designated as in Figure 1.

The distribution of the Hr 90-sample has a small shoulder on its low molecular part. The shape of the bimodality is changed during the delignification and at the end of the cook, the low molecular weight component of the residual lignin dominates, and the remaining high molecular portion of Hr 210 appears as a shoulder. In the THF-system, Figure 5, the MWD of acetylated residual lignin samples corresponding to the beginning and end of the bulk delignification phase are also bimodal. At the end of the bulk phase the residual lignin (AcHr 210) becomes fairly unimodal. A unimodal MWD of acetylated residual birch lignin corresponding to our AcHr 210-sample have been reported,[22] using ultrastyragel columns and THF as mobile phase. Hortling et al.[7] found a bimodal distribution of lignin leached by alkaline from batch and flow-through cooks of birch kraft pulp. The MWD of underivatized lignin was obtained using Fractogel TSK and an alkaline eluent. By enzymatic treatment prior to leaching, the amount and molecular weight of the lignin were increased, but the bimodality of the chromatograms remained.

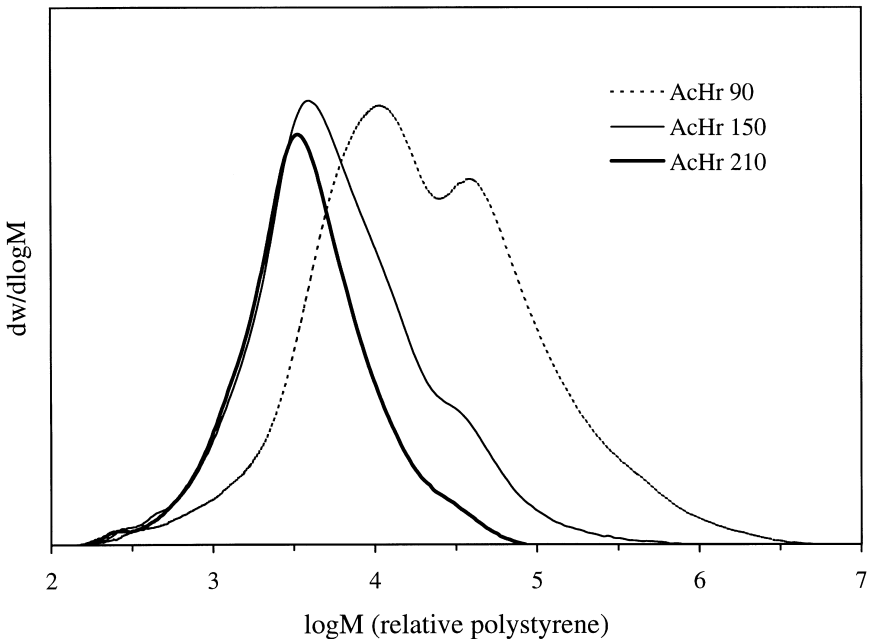

Figure 5. Molecular weight distributions of acetylated residual lignin samples isolated from kraft birch pulps of different degrees of delignification. Chromatographic conditions are as in Figure 3. Samples are designated as in Figure 1.

Limitations in Using Lithium Chloride/N,N-Dimethylacetamide

One striking difference between the two solvents is the system peak present when LiCl/DMAc is used as eluent. The system peak, mainly caused by LiCl, interferes with the low molecular weight portion of the dissolved lignin, making it impossible to evaluate the MWD of the whole sample (Figure 2). In contrast, in the THF-system no such interference occurred (Figure 3). The evaluation of the dissolved lignin samples characterised in LiCl/DMAc, could not be improved by the use of an UV-detector. The mobile phase contains an UV-absorbing component (DMAc) which causes a system peak, which also interferes with the low molecular weight portion of the dissolved lignin samples. In the LiCl/DMAc-system, the columns were chosen in order to cover the elution range of relatively high-molecular weight hemicelluloses and residual lignin, but are obviously not sufficient when characterising low-molecular weight lignin samples. An additional HR0.5 column may be sufficient to separate the system peak from the low-molecular portion of dissolved kraft lignin samples.

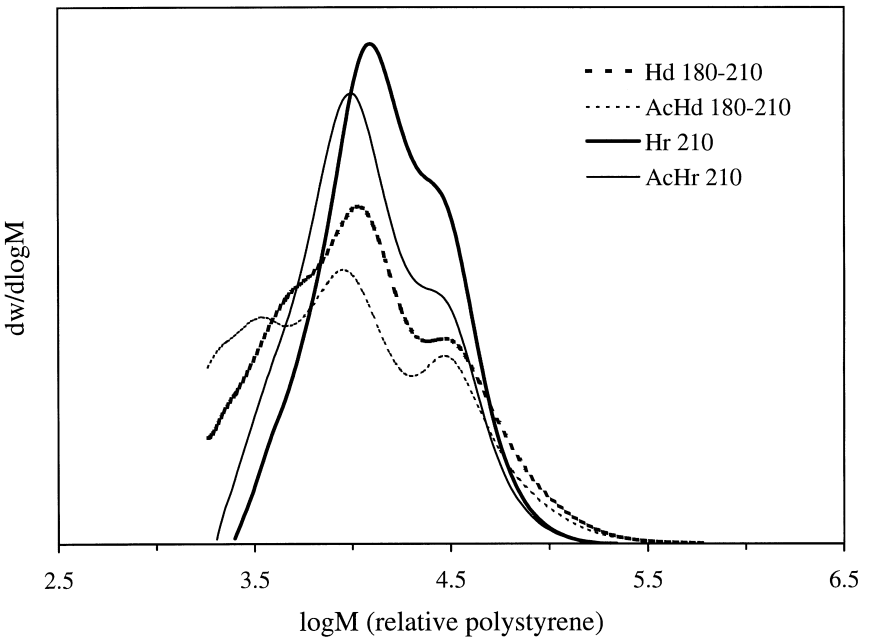

Figure 6. Influence of acetylation on the molecular weight distributions of dissolved and residual lignin samples corresponding to the end of cooking. Ac = acetylated sample, other samples are underivatized and are designated as in Figure 1. Chromatographic conditions are as in Figure 2.

Influence of Acetylation

Since LiCl/DMAc dissolves both underivatized and acetylated samples, the influence of acetylation on the MWD was studied. In Figure 6, the MWDs of underivatized and acetylated dissolved and residual lignin obtained at the end of the cook are shown. Acetylation was found to decrease the hydrodynamic size of lignin of all samples. In addition, the highest molecular weight sample, Hr 90, also showed an increase in the high molecular weight region upon acetylation (Figure 7). The logarithmic scale of the molecular weight complicates the interpretation of the apparent strong shift of the lower MWDs of the studied lignin samples to a lower molecular weight range. Nevertheless, one reason may be that the lignin samples consists of fragments of higher phenol content in the lower molecular weight range. The tailing to higher molecular weight range of AcHr 90, may indicate a higher concentration of phenol groups present in the largest lignin fragments of this sample.

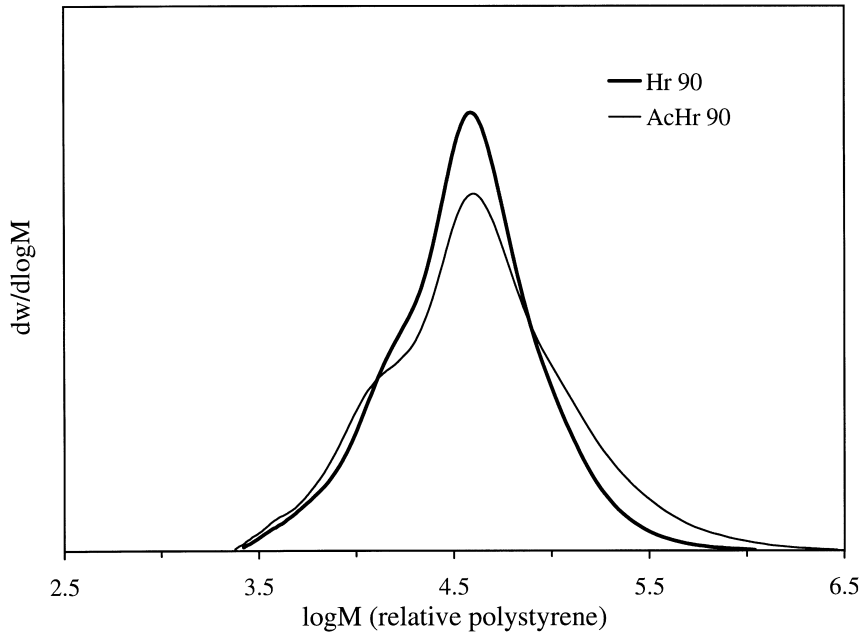

Figure 7. Influence of acetylation on the molecular weight distribution of residual lignin corresponding to the beginning of the cook. Hr 90 = underivatized and AcHr 90 = acetylated sample. Chromatographic conditions are as in Figure 2.

Due to the poor solubility in THF, the influence of acetylation has only been reported for low molecular weight lignin samples. The molecular weight of organosol hardwood lignin is decreased when acetylated,[10] whereas acetylation of explosion wood lignin of birch[32] and lignin samples originating from softwood increases the molecular weight.[32,33]

In Figure 8, the influence of derivatization and solvent on the MWD of a residual lignin sample is illustrated. Acetylation of the sample slightly decreased the apparent molecular weight but the shape of the distribution was essentially unaffected when using LiCl/DMAc as solvent.

In contrast, the choice of solvent considerably influenced the apparent molecular weight and the shape of the distribution. In LiCl/DMAc, the apparent molecular weight of the lignin is higher, the distribution is bimodal and narrower compared with the distributions obtained with the THF-system.

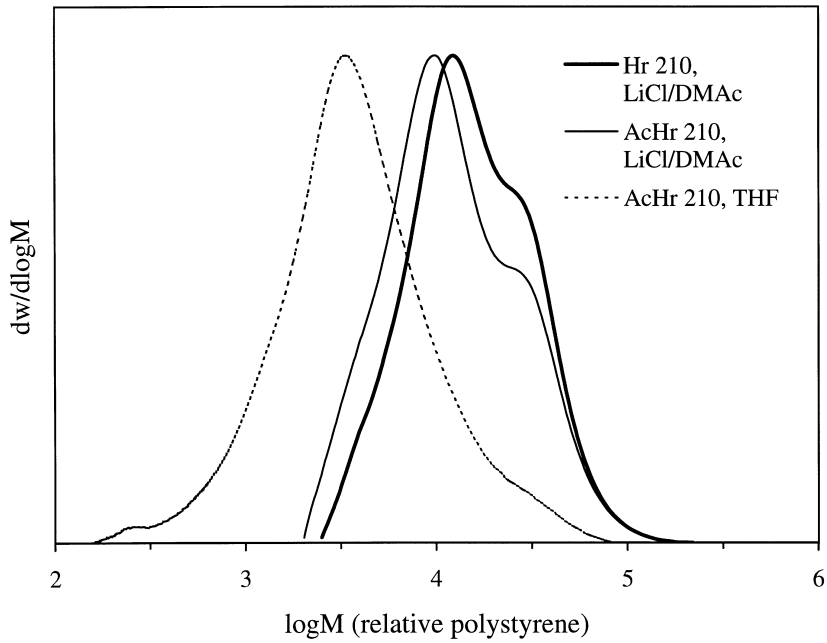

Figure 8. The influence of derivatization and solvent on the molecular weight distribution of a residual lignin sample isolated from a pulp after a completed cook. Characterisations were performed on three HR-columns using 0.5% LiCl/DMAc as mobile phase and on three ultrastyragel columns using THF as mobile phase, respectively.

Detector Response

As shown in Figures 3 and 4 the signal of the differential refractive detector increased with increased molecular weight of the residual lignin samples for both LiCl/DMAc and THF. The response of the refractive index detector may vary for different structures. Structural heterogeneity with respect to methoxyl and phenol groups in different molecular ranges has been reported for birch lignin isolated from black liquor[34] and organosol pulping of birch.[35] In addition, the dn/dc for a given polymer changes with increasing molecular weight up to a critical molecular weight that is specific for the polymer-solvent system.[36] For polystyrenes the asymptotic value of dn/dc is reached in the molecular weight range $2.0 \cdot 10^4$–$2.5 \cdot 10^5$ depending on solvent.[36] Due to the heterogeneous nature of lignin, a corresponding study is not possible to do. The lignins used in our study may be within the critical range where the response in different parts of the chromatogram may vary due to differences in molecular

weight and/or molecular structure. For lignin samples in the molecular weight range $1.9 \cdot 10^4$–$4.8 \cdot 10^4$ a concomitant increase in dn/dc and molecular weight have been reported.[37] The samples were isolated by acid dioxane extraction of spruce sawdust for various times. The amount of methoxyl groups was about the same in all samples, which indicates that the difference in dn/dc is not due to structural differences.

Molecular Weight Relative Polystyrene

The columns used in this study were packed with styrene-divinylbenzene, differing only in particle and pore size. The polarity of the solvent affects the pore size of the gel bed and the hydrodynamic radius of polystyrene and of the lignins. Using μ-Spherogel columns, Chum et al.[10] found that as the polarity of the solvent increases, polystyrene is eluted at longer retention times. This was assigned to interactions between the aromatic moiety and the styrene-divinylbenzene matrix of the column. Since the aromatic rings are substituted in lignins, interactions between lignin and the column packing may not be as pronounced as for polystyrene. Although polystyrene is a linear homopolymer, it is frequently used for molecular weight calibrations in lignin characterisations. It can be assumed that the hydrodynamic volume of the standards and the samples are different, and that only a relative molecular weight is obtained for lignin samples. In the LiCl/DMAc-system used in this study, all of the lignin samples have an apparent higher molecular weight compared to those obtained by the THF-system. This is most evident for the residual lignin samples (Figures 4 and 5). Interactions between the solute and the column packing do not seem to be of major importance when LiCl/DMAc is used as solvent. On the other hand, THF has a Hildebrand solubility parameter close to that of styrene[38] and consequently adsorption onto the polystyrene-divinylbenzene gel ought to be minimized when THF is used. It is difficult to assign a single explanation for the observed difference in molecular weight between LiCl/DMAc and THF. The reason may be explained by a combination of the following factors:

The polystyrene standards interact with the polystyrene-divinylbenzene matrix of the column when LiCl/DMAc is used as mobile phase.

The conformation of the lignin macromolecule is more extended i.e., have a larger hydrodynamic radius in LiCl/DMAc compared to when dissolved in THF.

The packing material of the column swells more in LiCl/DMAc than in THF. Consequently, the size of the pores becomes smaller in LiCl/DMAc making fewer pores accessible to the sample molecules. A

prerequisite for the apparent higher molecular weight obtained with LiCl/DMAc is that the polystyrene standards adopts a different conformation, and thereby cannot compensate for the decrease in the number of available pores.

An extensive association between lignin fragments occurs in LiCl/DMAc. Lignins are most soluble in solvents having a Hildebrand solubility parameter around 11.[1] For neat DMAc the value is 10.8 H and for THF 9.3 H.[38] The lignin samples were easily dissolved in DMAc. But just as reported for dimethylformamide (DMF),[9] the samples showed extensive association in absence of salt. Since addition of 0.1-1 mM LiCl to DMF was sufficient to disrupt association between lignin fragments,[9] the concentration of LiCl used in this study, about 0.1 M (0.5%), should be sufficient to avoid intermolecular associations.

The Shape of the Molecular Weight Distributions

The bimodal distribution of the residual lignin samples in this study may reflect the structure of the residual lignin at different degrees of delignification or modification of the lignin structure during isolation. The interpretation is complicated since the bimodality is differently accentuated in the two chromatographic systems used. For example, Hr 90 has a high amount of syringyl lignin and low concentration of phenol groups. When characterised using LiCl/DMAc, the bimodality is least pronounced, whereas the same sample shows a clear bimodality in THF. The difference in the shapes of the MWDs may be due to the following explanations:

Different parts of the chromatogram represent different structures that interact with the solvent in different ways depending on the polarity of the solvent.

Associations between lignin fragments cause the bimodality. If this is the case the apparent molecular weight and/or structure of the residual lignin samples and the polarity of the solvents are crucial. All distributions of the residual lignins obtained by the LiCl/DMAc-system have a larger portion of the sample in the high molecular weight fraction of the bimodality compared to when chromatographed in THF. Consequently, this would imply that associations between lignin fragments are pronounced even though a high concentration of salt is used which is unlikely.

Irrespective of whether the samples are derivatized or not, or the solvent used, the MWD of dissolved lignin samples in this study shifted towards higher molecular weight range, whereas, the MWD of the residual lignin samples

shifted to a lower molecular weight range during the cook. The opposite change in molecular weight has been observed for residual lignins isolated from spruce kraft pulps of different degrees of delignification.[26] The relative large molecular weight of the residual lignin in the beginning of the cook and the gradual decrease in the molecular weight of the residual lignins presented in this study may indicate that the size of the molecule influences the delignification of birch kraft pulps. In order to remove the lignin from birch pulp during kraft cooking, it seems that the lignin polymer have to be considerably degraded.

At the end of the cook, the MWD of residual and dissolved lignins seems to be quite similar (Figure 6). The observed similarity in the molecular size of dissolved and residual lignin at the end of flow-through kraft cooking of birch is in agreement with equivalent samples obtained from batch cooking of pine.[39]

CONCLUSIONS

The characteristics of dissolved lignin samples isolated from flow-through kraft cooking of birch are similar to those obtained from interrupted batch cooking. According to the characteristics of the residual lignin samples, acid dioxane extraction can be used to isolate residual lignin from birch pulps with high lignin content, although a certain degradation cannot be ruled out. The observed change in molecular size of the dissolved and residual lignin samples during kraft pulping of birch, may indicate that the large size of the residual lignin is decisive for the removal of lignin during the bulk delignification, but at the end of the cook no such restriction remains.

Using LiCl/DMAc as solvent, both underivatized and acetylated lignin samples can be characterised by size exclusion chromatography. However, low molecular weight samples cannot be fully evaluated with the applied LiCl/DMAc-system because of interference with the salt peak. The influences of acetylation on the MWDs indicate that the phenol groups of the lignin samples are not evenly distributed in the entire molecular weight range.

The reason for the apparent larger hydrodynamic size of lignin observed in the LiCl/DMAc-system compared to the THF-system is probably a combination of several factors. In LiCl/DMAc, the polystyrene standards could interact with the column matrix, the lignin molecules may be more extended and/or the packing material is more swelled compared with the THF-system. The differently accentuated bimodality in the MWDs obtained for the residual lignin samples chromatographed in LiCl/DMAc or THF may reflect that these lignins contain structures that interact differently depending on the polarity of the solvent.

The fundamental reason for the apparent bimodality may thus be due to structural differences in different molecular weight ranges. If this corresponds to the structure of the pulp lignin or if it is due to modifications during isolation is unclear. To exclude that an association between lignin fragments is responsible for the bimodality, further work is required.

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