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

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

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**ABSTRACT**

Dissolved and residual pine kraft lignin samples were isolated from different delignification phases of kraft flow-through cooks. The molecular weight distributions (MWDs) were studied by size exclusion chromatography using lithium chloride/N,N-dimethylacetamide (LiCl/DMAc), not previously applied to isolated softwood lignin samples before. The MWDs were compared with results obtained using tetrahydrofuran (THF) or lithium chloride/N,N-dimethylformamide (LiCl/DMF) as mobile phase. Some important characteristics of the isolated lignin samples including the MWDs are reported and compared with a previous study on birch wood kraft lignins.

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The molecular weight of the lignins was changed in a similar way irrespective of mobile phase used. The molecular weight of the dissolved lignin increased during the cook. By contrast, the change in molecular weight of the residual lignin samples did not show a clear trend with respect to cooking time. One explanation for this irregular change may be the low efficiency of the acid dioxane extraction of the pine kraft pulp obtained early in the cook as this pulp contains a large amount of lignin. Apart from this sample, the size of the residual lignin sample decreases and resembles that of the dissolved lignin at the end of the cook. For all samples, the MWD was positioned at higher molecular weight range when LiCl/DMAc was used as the mobile phase compared with THF.

The explanation for this behaviour seems to be that the polystyrene standards elutes later from polystyrene-divinylbenzene columns compared to the lignin samples when a mobile phase of higher polarity is used. The shapes of the distributions were also different between LiCl/DMAc and THF, whereas LiCl/DMAc and LiCl/DMF gave similar distribution profiles. These results emphasize the importance of using the same mobile phase and column packing material when comparing MWD and size of different lignins and also when comparing lignins and carbohydrate polymers in pulp.

## INTRODUCTION

Lignin is one of the most abundant polymers in plants. In spite of this, the precise structure of lignin is not completely known. The principal structure of softwood lignins is methoxyhydroxyphenylpropane (guaiacyl) units. More than two thirds of these units are linked by ether bonds and the remainder by carbon-carbon bonds.[1] In addition to aliphatic hydroxyl groups, the structure includes other functional groups such as methoxyl, carbonyl, and phenol groups in various amounts, depending on morphological origin.[2,3] During kraft processing of wood most of the lignin is removed from the pulp, and the main portion can be recovered as polymeric lignin from the black liquor. The delignification during kraft pulping is governed by the amount of accessible reactive structures in lignin,[4,5] the hydrophilicity,[4,6] and by the size of the lignin fragments.[7]

In order to obtain information about the molecular weight distribution (MWD), lignins are routinely characterised by Size Exclusion Chromatography (SEC). A number of solvents have been used as mobile phase for characterisation of underivatized and derivatized lignin samples; examples are given in Part 1 of this paper.[8] The method most commonly used to characterise acetylated lignins employs tetrahydrofuran (THF) as mobile phase.

To directly compare the MWD of isolated lignins with the lignin and carbohydrate polymers remaining in the pulp, it would be an advantage to use the same solvent system during characterisation.  Whereas the traditional lignin solvents do not dissolve wood pulps, lithium chloride/N,N-dimethylacetamide (LiCl/DMAc) completely dissolves hardwood kraft pulps.[9]  In contrast to hardwood kraft pulps, softwood kraft pulps are not completely soluble in LiCl/DMAc.[10,11]  The solubility of softwood kraft pulps varies between 70 - 90%, depending on the degree of delignification and on gel formation due to the glucomannan present in the pulp.[11]  Still, LiCl/DMAc has also been used for the characterisation of softwood kraft pulps.[10,12]

In a previous study, lignin samples isolated from kraft cooks of birch were characterised by SEC using LiCl/DMAc as mobile phase.[8]  In this work, the applicability of using LiCl/DMAc as mobile phase in SEC characterisation of dissolved and residual lignin samples isolated from flow-through kraft cooks of pine is demonstrated.  One of the residual lignin samples was isolated from a partly defibrated pine wood pulp.  This type of residual lignin sample has not been isolated before by acid dioxane extraction.  Some important characteristics of the obtained lignin samples including the MWDs are reported and compared with the previous report on a corresponding study of birch wood lignins.  The influence of mobile phase composition on the chromatographic behaviour and MWDs relative polystyrene is studied by comparing LiCl/DMAc with lithium chloride/N,N-dimethylformamide (LiCl/DMF), and tetrahydrofuran (THF), respectively.

## EXPERIMENTAL

### Materials

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); N,N-dimethylacetamide, and N,N-dimethylformamide were purchased from Sigma-Aldrich (Gillingham, UK).

### Cooking Conditions and Sample Preparation

The abbreviations used for wood, pulp, and isolated lignin samples are detailed in Table 1.  Dry pine (*Pinus Sylvestris*) chips were impregnated with deionized water before flow-through kraft cooks were performed.  Cooking conditions were essentially equal to those described for birch wood.[8]  For pine wood, the initial temperature was 70°C, which was increased by 1°C/min to a final temperature of 170°C.  After the cook, the pulps were washed thoroughly and viscosity and lignin content measured.
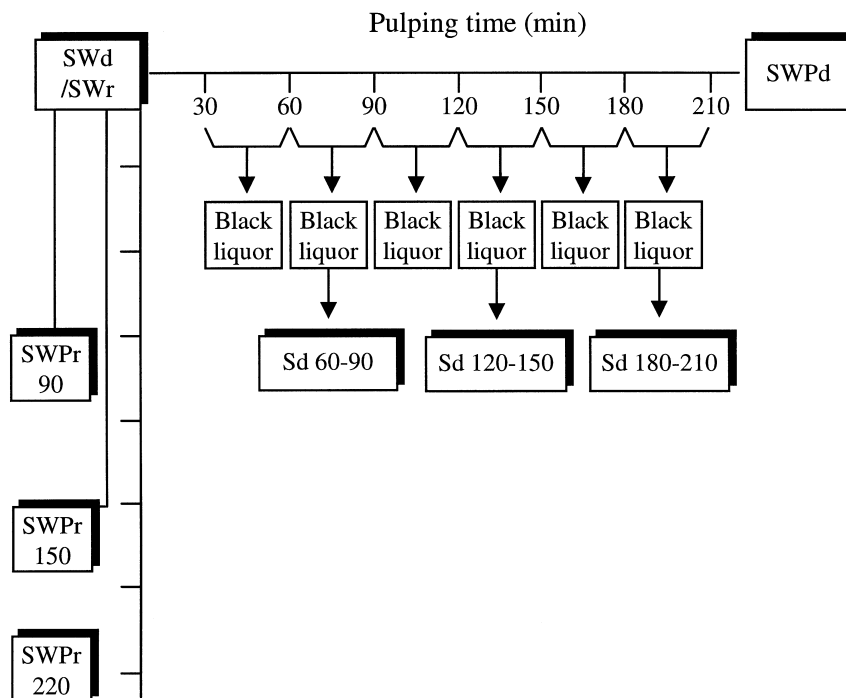
**Figure 1.** An overview of wood, pulps and black liquor obtained from flow-through kraft cooking of pine wood. The time range refers to the collection time of black liquor from which the sample was isolated. Residual lignin samples Sr 90, Sr 150 and Sr 220 were isolated from the pulps designated SWPr. The numbers given together with the abbreviation refer to the cooking times. Abbreviations are according to Table 1. Sd = pine wood dissolved lignin.

**Table 1**

**Abbreviations for Wood,Pulp and Isolated Lignin Samples**

| | |
|---|---|
| SWd | Softwood chips cooked to obtain dissolved lignin |
| SWPd | Softwood pulp obtained from cooking of SWd |
| Sd | Dissolved lignin samples obtained from cooking of SWd |
| SWr | Softwood chips cooked to obtain residual lignin |
| SWPr | Softwood pulps obtained from cooking of SWr |
| Sr | Residual lignin samples obtained from cooking of SWr |

Black liquor was continuously collected in six consecutive fractions (see Figure 1). Dissolved lignin was isolated from three of the fractions by a procedure described in detail in Ref. 8. Residual lignin was isolated from pulps originating from three different flow-through kraft cooks which were stopped after 90, 150, and 220 minutes, respectively (see Figure 1). The 90-minute cook rendered the wood chips only partially defibrated. To increase the accessibility of fibre during isolation, this pulp was milled to 4 mm granules prior to further treatment. After acetone extraction, residual lignin was isolated from the pulps by acid dioxane extraction at 88°C according to the method of Gellerstedt et al.[13]

## Characterisation of Wood and Pulp

Pulps are commonly characterised by the limiting viscosity number [η] after dissolution in cupriethylenediamine, which was determined according to SCAN-CM 15:88.[14] The limiting viscosity number [η] is defined by the expression

$$[\eta] = \lim(c \to 0)\ (\eta - \eta_0)/\eta_0 c$$

where $\eta$ is the viscosity of the test solution and $\eta_0$ that of the solvent and c the concentration of the test solution in g/mL. The relative viscosity, $(\eta - \eta_0)/\eta_0$, is defined by

$$(\eta - \eta_0)/\eta_0 = (t - t_0)/t_0$$

where t and $t_0$ are the efflux times measured for a specified volume of test solution and solvent, respectively, in two separate capillary viscometers. Kappa number is used to describe the degree of delignification obtained in a chemical pulping process. It is defined as the number of mL of 20 mM potassium permanganate solution consumed by one gram of moisture-free pulp and was determined according to SCAN-C 1:77.[15] Gravimetric determination of acid insoluble lignin (Klason lignin) was conducted following hydrolysis of wood and pulp, respectively, in 82% sulphuric acid at room temperature for two hours, followed by dilution to 3% and autoclave treatment at 120°C for an additional half hour.

## Characterisation of Lignin Samples

Elemental composition and ash content were determined by Mikro Kemi AB, Uppsala, Sweden and methoxyl analysis was performed by Analytische Laboratorien, Elbach, Germany. The concentration of phenol groups was determined by aminolysis at STFI according to the procedure described by Månsson.[16]

### Size Exclusion Chromatography of Lignin Samples Using 0.5% LiCl/N,N-Dimethylacetamide or LiCl/N,N-Dimethylformamide as Mobile Phase

In each run, 50 µg of underivatized lignin sample was 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 35°C on three columns connected in series, HR5E, HR1, and HR0.5 (Waters), using a flow rate of 1 mL/min. The columns were calibrated with polystyrene standards, in the range 2930 D to 2.56 MD (Polymer Laboratories Ltd., UK). Cubic coefficient of determination ($r^2$) between the peak molecular weight ($M_p$) of the standard and the time scale was 0.993 for LiCl/DMAc and 0.997 for LiCl/DMF. Data acquisition and calculations were carried out using PL Caliber (Polymer Laboratories Ltd., UK). Polystyrene standards in the range 580 D to 350 kD (Polymer Laboratories Ltd., UK) were also analysed at room temperature, using tetrahydrofuran (THF) as mobile phase. The cubic coefficient was 0.992. The columns for LiCl/DMAc, were equilibrated for one day in LiCl/N,N-dimethylformamide before use and for four days in THF when this solvent was to be used as mobile phase.

### Size Exclusion Chromatography of Lignin Samples Using Tetrahydrofuran as Mobile Phase

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, $10^4$Å, 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. Polystyrene standards in the range 580 D to 350 kD (Polymer Laboratories Ltd., UK) were used to calibrate the columns. The cubic coefficient between the $M_p$ and time was $r^2 = 1.00$. Data acquisition and calculations were carried out using Baseline (Waters).

### RESULTS

### Characteristics of Pulp and Lignin Samples

Lignin dissolved in black liquor was isolated from fractions continuously collected during a flow-through kraft cook of pine wood. To obtain residual lignin samples, i.e. the lignin remaining in the pulp, from different phases of the cook, three additional cooks were performed. In Figure 1 an overview of the wood, pulp, and black liquor used to obtain the lignin samples is shown. The samples correspond to different delignification phases in the kraft cook.[17] The transition from initial to bulk delignification phase occurs during the 60-90 minutes interval. The samples obtained during the 150-180 minutes interval

**Table 2**

**Pulp Yield\*, Viscosity, Degree of Delignification, and Lignin Content of
Pulps Used for Isolation of Dissolved and Residual Lignin**

| Sample[a] | Pulp Yield (%) | Viscosity (mL/g) | Degree of Delignific. (%) | Kappa Number After Cooking | Extraction[b] | Klason Lignin (%) |
|---|---|---|---|---|---|---|
| SWd | --- | --- | --- | --- | --- | 26.8 |
| SWPd 210 | 44.8 | 1200 | 90.3 | 22 | --- | 2.6 |
| SWr | --- | --- | --- | --- | --- | 25.9 |
| SWPr 90 | 69.7 | --- | 19.7 | 83 | 76 | 20.8 |
| SWPr 150 | 49.1 | 1370 | 81.1 | 39 | 18 | 4.9 |
| SWPr 220 | 45.1 | 1160 | 92.7 | 16 | 6.9 | 1.9 |

\* = % of wood.  [a]Designation according to Table 1.  [b] Acid dioxane extraction.

represents the end of the bulk delignification phase.  After about 180 minutes the delignification rate decreases.  The pulp and black liquor samples obtained during this final part of the cook correspond to the residual delignification phase.

The pulp yield, pulp viscosity, degree of delignification, and lignin content of wood and pulps are shown in Table 2.  The most accurate way of estimating the lignin content in a pulp is to measure the amount of acid insoluble residue, i.e. Klason lignin.  In order to compare the efficiency of the isolation method of residual lignin with the previous study on birch wood kraft pulps,[8] kappa numbers of pulps before and after acid dioxane extractions are reported in Table 2.  The kappa number is a measure of all structures oxidised by potassium permanganate, i.e. it also includes unsaturated structures derived from the carbohydrates,[18] but is commonly used as a rough estimate of the amount of lignin in pulp.

Although different batches of pine wood chips were used the yield and viscosity of the fully cooked pulps, SWPd 210 and SWPr 220, are similar.  The degree of delignification was calculated from the lignin content in the wood and pulps determined as Klason lignin.  To avoid severe degradation of the carbohydrate polymers, kraft cooks are generally stopped when about 90% of the lignin has been removed.  The difference in lignin content between the two fully cooked pulps is due to the difference in lignin content between the two batches of chips and longer cooking time for the SWPr 220-pulp compared to the SWPd 210-pulp.  Of the lignin dissolved during the cook, 59% (calculated from Klason lignin) was recovered by acid precipitation.

**Table 3**

**Normalised Molecular Formula and Concentration of Phenol Groups
of Lignin Samples**

| Sample[a] | Molecular Formula | Phenol Groups mmol/g |
|---|---|---|
| Sd 60-90 | $C_{100}H_{100}N_{0.3}O_{28.0}S_{4.3}(OCH_3)_{9.0}$ | 2.6 |
| Sd 120-150 | $C_{100}H_{96}O_{28.6}S_{2.2}(OCH_3)_{9.0}$ | 2.7 |
| Sd 180-210 | $C_{100}H_{96}O_{28.0}S_{3.0}(OCH_3)_{8.6}$ | 3.0 |
| Sr 90 | $C_{100}H_{91}O_{28.1}S_{0.38}(OCH_3)_{10}$ | 2.2 |
| Sr 150 | $C_{100}H_{95}O_{26.4}S_{0.64}(OCH_3)_{8.6}$ | 2.7 |
| Sr 220 | $C_{100}H_{91}N_{0.3}O_{26.0}S_{0.79}(OCH_3)_{9.0}$ | 3.2 |

[a] Designation according to Table 1.

The remaining lignin released in the black liquor consists of low molecular weight hydrophilic fragments, not possible to isolate by the applied method. Based on the amount of Klason lignin, about 50% of the residual lignin was recovered by acid dioxane extraction of SWPr 150 and SWPr 220, whereas only about 13% was recovered from the partly defibrated SWPr 90-pulp.

To characterise the prepared samples, some fundamental characteristics were determined. The molecular formula of each sample was calculated from elemental analysis and methoxyl group determination according to Zakis, 1994.[19] Molecular formulas, normalised to $C_{100}$, and the concentration of phenol groups are shown in Table 3. The methoxyl content was similar in all of the lignin samples except for the residual lignin sample isolated at the beginning of the bulk delignification phase, which had a higher content. The concentration of phenol groups increased in both dissolved and residual lignin samples as the extent of delignification increased.

Apart from the lignin samples corresponding to the beginning of the bulk delignification phase, Sd 60-90 and Sr 90, respectively, the concentrations of phenol groups in the dissolved and residual lignin samples are about the same at any given cooking time. The nitrogen, present in the dissolved lignin sample isolated early in the cook, indicates a low contamination by protein. As commonly seen in lignin samples from kraft cooking, all samples also contain sulphur as a result of lignin reactions with the hydrogen sulphide ions used as delignification agent. The dissolved lignin samples contain larger amounts of sulphur than the residual lignin samples and this is in agreement with earlier studies of birch wood kraft lignins.[8,20]

## Molecular Weight Distribution

Two chromatographic set-ups were used to characterise the lignin samples. Both set-ups were equipped with a refractive index detector and polystyrene-divinylbenzene columns differing only in particle and pore size.  In one of the systems, 0.5% LiCl/DMAc or 0.5% LiCl/DMF was used as the mobile phase. In the alternative system only THF was used.

All lignin samples, underivatized or acetylated, were completely soluble in 0.5% LiCl/DMAc.  To obtain complete dissolution in THF all samples were acetylated.  Ac is used as a prefix to designate acetylated samples throughout this study.  Acetylation of lignin samples only slightly influenced the results when using LiCl/DMAc;[8] hence only the results for underivatized samples are presented here for LiCl/DMAc.

The apparent MWDs of dissolved lignin samples are shifted towards a higher molecular weight range when LiCl/DMAc is compared with the THF-system, Figure 2.  Another difference between the two chromatographic systems concerns the shape of the distributions.

In the LiCl/DMAc-system, the sample obtained at the end of the initial delignification phase, Sd 60-90, has four discernible maxima of which one is more pronounced.  As the delignification proceeds, the two maxima in the low molecular weight range grow less pronounced and the maximum of highest molecular weight increases.

In the THF-system, the AcSd 60-90 has two clearly discernible maxima. In addition, its MWD is positioned in a much lower molecular weight range compared with the samples obtained during the bulk delignification phase.  The MWDs of AcSd 120-150 and AcSd 180-210 were similar, having one main maximum and one shoulder on each side of the distributions.
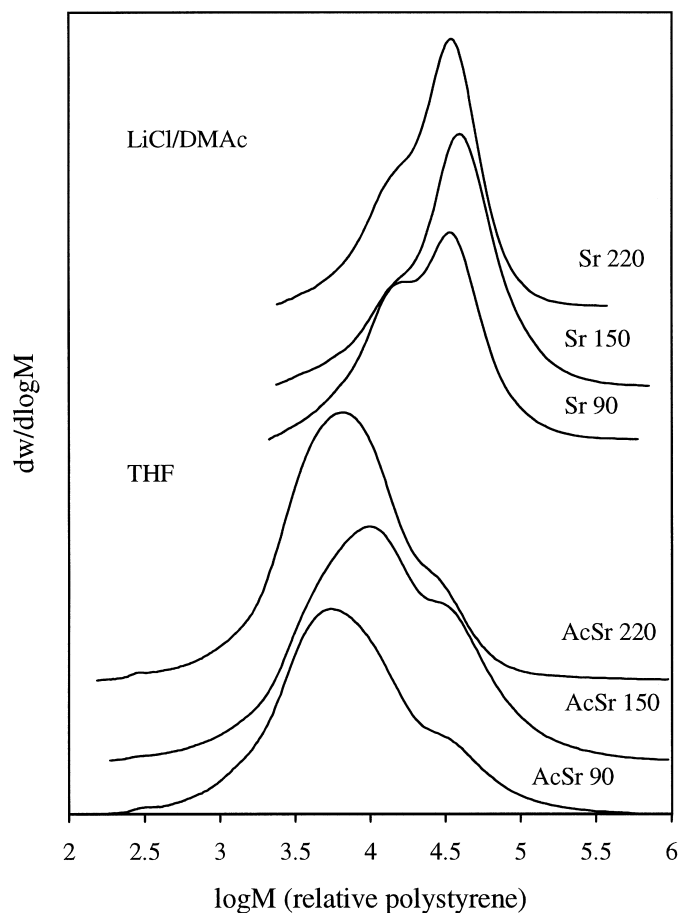
In Figure 3, MWDs of lignin samples isolated by acid dioxane extraction of pulps, i.e. residual lignin is shown. As for the dissolved lignin samples, the apparent MWDs are positioned in a much higher molecular weight range when using the LiCl/DMAc-system than when using the THF-system.  Irrespective of chromatographic system used, the molecular weight decreases in the following order: (Ac)Sr 150>(Ac)Sr 90>(Ac)Sr 220.  All distributions are bimodal but the relative intensity of the peaks is different depending on the choice of mobile phase.

In the LiCl/DMAc-system, the distribution of the highest molecular weight portion dominates in all residual lignin samples whereas in the THF-system the distribution of the lowest molecular weight portion is the major peak. The most resolved bimodality in the LiCl/DMAc-system is obtained for the Sr 90 sample whereas in the THF-system, bimodality is most pronounced for Sr 150.
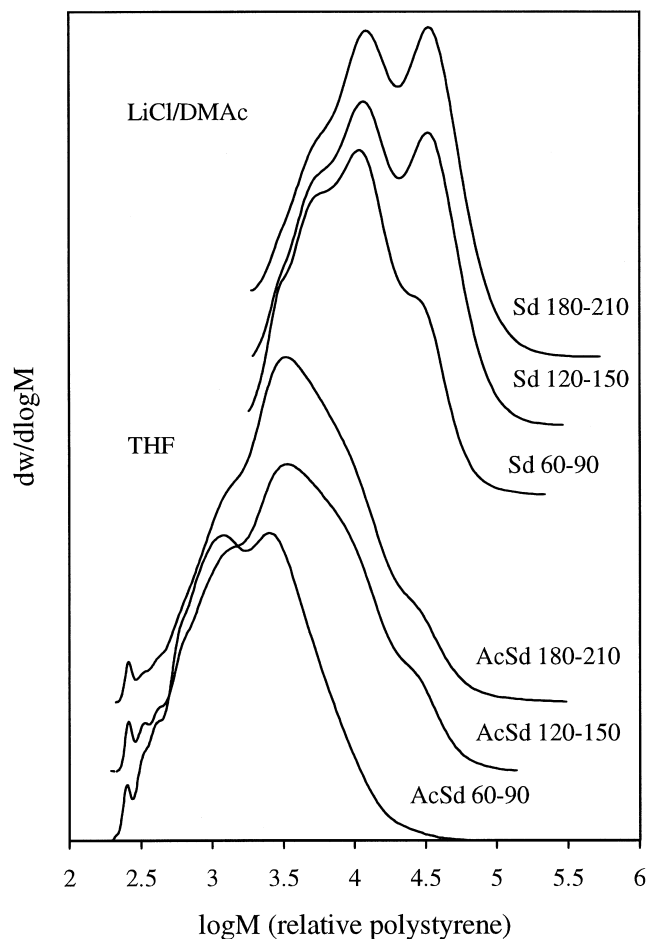
**Figure 2.** Molecular weight distributions of underivatized and acetylated dissolved lignin samples isolated from black liquor collected during a kraft cook of pine wood. The underivatized samples were chromatographed on a series of three HR-columns using 0.5% LiCl/DMAc as mobile phase and the acetylated samples were chromatographed on a series of three ultrastyragel columns using THF as mobile phase. Samples are designated as in the legend in Figure 1, the Ac-prefix corresponds to acetylated samples.

During delignification, the MWD of the dissolved lignin is shifted gradually to a higher molecular weight range and becomes more equal to that of the residual lignin. At the end of the cook, the highest molecular weight portion of the dissolved lignin sample coincides with the main portion of the residual lignin sample.

**Figure 3.** Molecular weight distributions of residual lignin samples isolated from kraft pine wood pulps of different degrees of delignification. The underivatized samples were chromatographed on a series of three HR-columns using 0.5% LiCl/DMAc as mobile phase and the acetylated samples were chromatographed on a series of three ultrastyragel columns using THF as mobile phase. Samples are designated as in the legend in Figure 1, the Ac-prefix corresponds to acetylated samples.

## Influence of Mobile Phase Composition on Molecular Weight Distribution

The MWD of a residual lignin sample (Sr 90) obtained by using the LiCl/DMAc-system was compared with that obtained by using 0.5% LiCl/N,N-dimethylformamide (LiCl/DMF) as mobile phase. The shape of the MWDs is the same for the two solvents (Figure 4), but the MWD of the lignin sample was
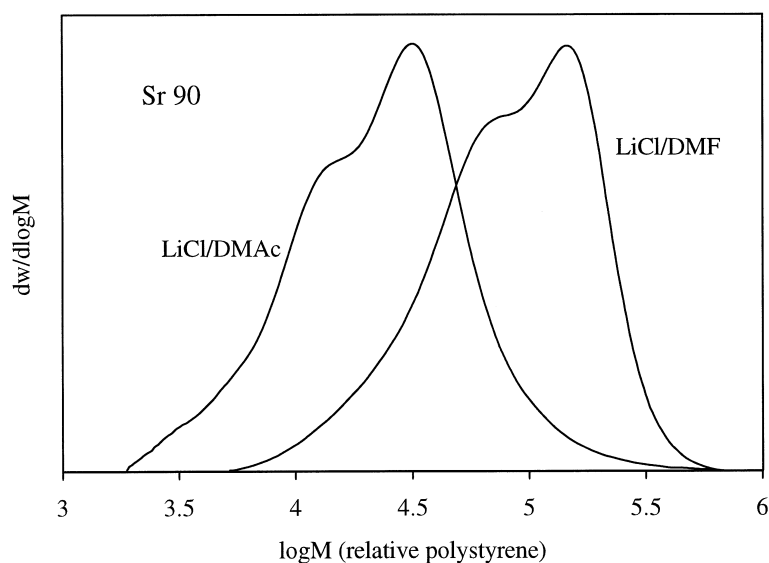
**Figure 4.** The influence of mobile phase composition on the molecular weight distribution relative polystyrene of an underivatized residual lignin sample (Sr 90). The sample was chromatographed on a series of three HR-columns using 0.5% LiCl/DMAc or 0.5% LiCl/DMF, respectively, as mobile phase.

positioned in a higher molecular weight range with LiCl/DMF as mobile phase than with LiCl/DMAc. For comparison, a calibration curve of polystyrene (PS) standards was also made with the same chromatographic set-up using THF as mobile phase. The calibration curves, shown in Figure 5, reveal the behaviour of PS standards in different mobile phases. As the polarity of the mobile phase increases, the PSs elute later, indicating interactions between PS and the gel matrix i.e. showing a non-ideal behaviour with respect to size-exclusion chromatography.

## DISCUSSION

Since the pulp characteristics are similar for the two fully cooked batches of pine wood, the dissolved lignin samples isolated from the black liquor can be compared with the residual lignin samples.

Klason lignin or kappa numbers determined before and after dioxane extraction of softwood kraft pulps, have only been reported in a few studies concerning residual lignin.
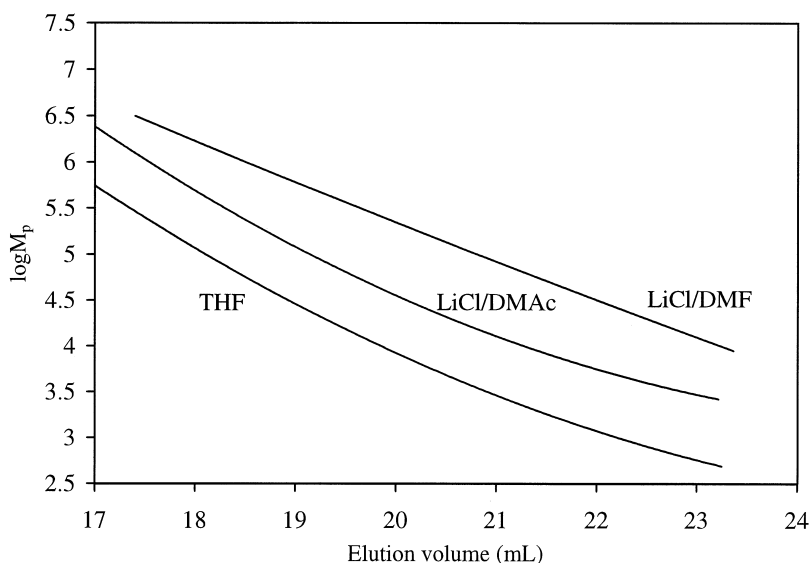
**Figure 5.** Calibration curves obtained by analysing polystyrene standards on a series of three HR-columns packed with polystyrene-divinylbenzene particles using different mobile phases. The cubic coefficient between $M_p$ and time was $r^2$ = 0.992 (THF), 0.993 (LiCl/DMAc) and 0.997 (LiCl/DMF).

The decrease in kappa number for the SWPr 220-pulp extracted by acid dioxane (this study) is about the same as reported by Froass et al.[21] for a similar pine wood kraft pulp. Using the same isolation method, Jiang and Argyropoulos[22] obtained lower yields of residual kraft lignin from black spruce wood delignified to about the same extent as the pulps SWPr 150 and SWPr 220 in this study. This is probably due to different sources of wood since the kappa numbers of the black spruce wood kraft pulps were much higher compared with SWPr 150 and SWPr 220. Acid dioxane extraction has not previously been used to isolate residual lignin from softwood pulps with high lignin content, such as SWPr 90. As indicated by the reduction in kappa number, only a small amount of residual lignin was released by this isolation method. This finding is in contrast with results obtained with acid dioxane-extracted birch wood kraft pulp of about the same degree of delignification where the kappa number was reduced from 84 to 27.[8] This may be explained by differences in lignin structure; hardwoods are also generally more easy to delignify than softwoods during kraft pulping.[17,23]

The amounts of methoxyl groups in the isolated lignin samples are in agreement with data published for dissolved lignin isolated from a flow-through kraft cook[24] and residual lignin samples isolated from batch kraft cooks.[21,22] As a

result of the cleavage of aryl-ether linkages during the cook, all of the lignin samples contain increasing amounts of phenol groups. The concentration of phenol groups was similar in the dissolved lignin samples and in the corresponding residual lignin samples. The concentration of phenol groups in the dissolved lignin samples in the present study is slightly lower than those reported by Robert et al.[24] The concentrations of phenol groups in the residual lignin samples SWPr 150 and SWPr 220 are higher compared with samples isolated from similar pine wood[21] and black spruce wood[22] kraft pulps. A comparison of different methods in the literature[25] shows that the method used in this study (aminolysis) is in agreement with the method used in Refs. 21 & 22 (^{31}P-NMR) and, therefore, cannot explain this difference. It should be noted that the residual lignin sample obtained at the end of the initial delignification phase (Sr 90) of the cook, has a relatively large amount of methoxyl groups and a low concentration of phenol groups as compared with other lignin samples. This may reflect the amounts of these functional groups in native lignin. However, since the Sr 90-sample only represents 13% of the pulp lignin, no definitive conclusion can be made from the above results.

As for birch wood kraft lignins,[8] the shapes of the MWDs are differently accentuated in LiCl/DMAc and in THF. It should be stressed that this difference is not dependent on sample acetylation. The dissolved lignin samples originating from the end of the bulk delignification phase and the residual delignification phase (Figure 2) have a clearly accentuated bimodality when LiCl/DMAc was used as the mobile phase. The gradual shift of the MWD of the dissolved lignins to higher molecular weights throughout the cook was most evident for LiCl/DMAc. The observed increase in molecular weight is in accordance with results published by others for softwood kraft lignins[24,26] and also for dissolved birch wood kraft lignin.[8] Dissolved lignin fragments having the lowest molecular weight could not be evaluated when LiCl/DMAc was used as mobile phase. This is due to the coelution of the salt, which affects the response of the refractive index detector. This drawback was more pronounced for dissolved lignins isolated from kraft cooks of birch wood,[8] because of their lower molecular weight compared with kraft lignins derived from softwood.

The molecular weight range of the residual lignin samples was changed in the same irregular way in both LiCl/DMAc and THF (Figure 3). This is in contrast to residual lignins isolated from kraft pulp of birch wood,[8] where the MWD of the residual lignin was shifted to a lower molecular weight range throughout the cook. One explanation for the apparent lack of continuous trend with regard to molecular weight in this study, may be the above-mentioned low efficiency of acid dioxane extraction of pine wood kraft pulps of high lignin content, i.e. SWPr 90, compared with the corresponding birch wood pulp. The residual lignin sample isolated from the end of the bulk delignification phase had the highest molecular weight. The decrease in molecular weight of the residual lignin samples originating from pulps with an increasing degree of delignification (81.1% to 92.7%), i.e., from the beginning of the bulk and residual delignification phase, respectively, may be due to degradation of the

pine wood lignin during the cook.  Jiang et al.[22] reported an increase in molecular weight of residual lignin with increasing degree of delignification. Their samples were isolated by acid dioxane extraction of black spruce wood kraft pulps with a degree of delignification of 39.8%, 71.3% and 92.5%.  The increase in molecular weight was ascribed to condensation reactions during kraft pulping.  The divergent changes in molecular weight for residual kraft lignin obtained at different degrees of delignification of pine wood, black spruce wood, and birch wood may indicate that acid dioxane extraction modifies the residual lignin samples differently depending on the species of wood.

Just as for the dissolved lignin samples, the shapes of the MWDs of the residual lignin samples obtained by LiCl/DMAc were different compared to when THF was used.  This was also observed for residual lignin samples originating from birch wood kraft pulps.[8]  The different shapes may indicate that each residual lignin sample consists of different lignin structures unequally distributed with respect to molecular weight.

At the end of the cook, the MWDs of dissolved and residual lignin become more equal.  This is in accordance with results reported by Gellerstedt et al.[27] who compared dissolved lignins from a flow-trough kraft cook with residual lignin isolated from a batch cook of pine wood.  Although more degraded, the major part of the MWD of the dissolved lignin sample resembles the bimodality of the residual lignin sample.

As observed for birch wood kraft lignins,[8] the MWDs relative PS are positioned in a higher molecular weight range when the elution is conducted with LiCl/DMAc from columns packed with polystyrene-divinylbenzene particles compared with elution by THF.  The reason for this behaviour may possibly be ascribed to interactions between the PS standards and the column matrix when LiCl/DMAc is used as mobile phase.  Alternatively, different degrees of interactions within the lignin polymer and thereby different conformations in the two solvents in combination with a higher degree of swelling of the polystyrene-divinylbenzene matrix of the column may contribute to this difference.

The influence of mobile phase composition on the size of lignin and PS standard was further studied by using 0.5% LiCl/ N,N-dimethylformamide (DMF) as mobile phase.  DMF containing lithium salts have earlier been used in SEC characterisation of different kinds of lignin samples.[28,29]  The results in the present study show that the MWD profile of lignin is the same whether LiCl/DMF or LiCl/DMAc is used as mobile phase.  Since the polarities of DMF and DMAc are similar, this observation suggests that the polarity of the mobile phase determines the shape of the MWD.  On the other hand, the residence time of the sample is longer when LiCl/DMF is used as mobile phase than when LiCl/DMAc is used.  Because smaller sized samples are eluted later than molecules of a larger size in SEC, this indicates that the hydrodynamic size of the lignin sample is smaller in LiCl/DMF than in LiCl/DMAc.  The longer

residence time of the lignin sample in LiCl/DMF may also be due to interactions between the sample and the polystyrene-divinylbenzene matrix and/or the pores of the separating particles becoming larger in LiCl/DMF.

The influence of the mobile phase on the molecular weight of lignin relative to PS standards was noticeable. In spite of the longer residence time obtained when LiCl/DMF was used as mobile phase, the molecular weight relative to PS was apparently higher compared with when LiCl/DMAc was used. According to the calibration curves, the polarity of the mobile phase influences the residence time of PS standards. As the polarity of the mobile phase increases, PS remains in the column for a longer time. This was also observed by Chum et al.,[29] and they suggested that this is due to adsorption onto the column matrix, but the swellability in different solvents may also contribute to this behaviour.

Taken together, although the residence time of both lignin and PS standards was prolonged when the polarity of the mobile phase increased, the PS standards were more affected and hence the molecular weights of lignin samples in relation to PS will be higher.

## CONCLUSIONS

This study has shown that the molecular weight of pine wood lignins are changed essentially in the same way during kraft cooking as was shown for birch wood lignin in a previous study.[8] The molecular weight of dissolved lignins increases and that of residual lignins gradually decreases during the cook. One exception was the residual lignin sample corresponding to the end of the initial delignification phase. This is probably due to the low efficiency of the acid dioxane extraction of undefibrated pulp that renders a lignin sample that is not representative for the pulp lignin.

The results emphasise the importance of using the same mobile phase and packing material in the columns to study changes in the shape of the MWDs during degradation as well as when comparing the size of lignin relative to carbohydrate polymers. Although the apparent molecular weights compared with PS standards was high when LiCl/DMAc was used as mobile phase, LiCl/DMAc still offers advantages as mobile phase for the characterisation of underivatized lignins.

## ACKNOWLEDGMENT

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