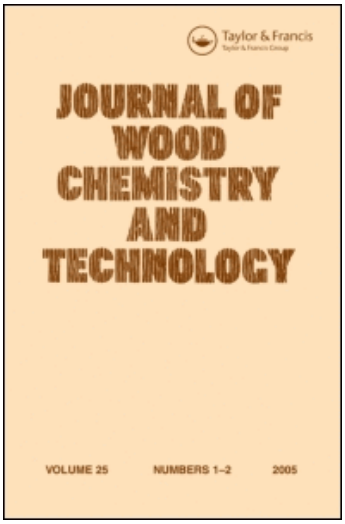

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

A Method for Evaluating Lignin Mobility Distributions Obtained by Capillary Zone Electrophoresis

Elisabeth Sjöholm[a]; Erik Norman[ab]; Störker Moe[c]; Anders Colmsjö[d]
[a] Swedish Pulp and Paper Research Institute, Stockholm, Sweden [b] Stora Enso Research, Karlstad, Sweden [c] Department of Chemical Engineering, Norwegian University of Science and Technology (NTNU), Trondheim, Norway [d] Arrhenius Laboratory, Department of Analytical Chemistry, University of Stockholm, Stockholm, Sweden

To cite this Article Sjöholm, Elisabeth , Norman, Erik , Moe, Störker and Colmsjö, Anders(2000) 'A Method for Evaluating Lignin Mobility Distributions Obtained by Capillary Zone Electrophoresis', Journal of Wood Chemistry and Technology, 20: 2, 113 — 132
To link to this Article: DOI: 10.1080/02773810009349628
URL: http://dx.doi.org/10.1080/02773810009349628

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 20(2), 113–132 (2000)

A METHOD FOR EVALUATING LIGNIN MOBILITY DISTRIBUTIONS OBTAINED BY CAPILLARY ZONE ELECTROPHORESIS

Elisabeth Sjöholm and Erik Norman[1]
Swedish Pulp and Paper Research Institute
Box 5604, SE-114 86 Stockholm, Sweden

Störker Moe
Norwegian University of Science and Technology (NTNU), Department of Chemical Engineering, Sam Saelands vei 4, N-7491 Trondheim, Norway

Anders Colmsjö
Arrhenius Laboratory, Department of Analytical Chemistry
University of Stockholm, SE-106 91 Stockholm, Sweden

ABSTRACT

The profile of the mobility distribution of lignin-containing samples depends on type of sample. To facilitate comparison, a procedure for determining the average mobility (μ_{av}), *i.e.* the average charge density, of lignin is presented. The procedure is applied to black liquor (Sb), isolated dissolved lignin (Sd) and isolated residual (Sr) lignin samples, obtained from flow-through kraft cooks of softwood. The μ_{av} of the isolated lignin samples is compared with the concentration of phenol and carboxyl groups and relative molecular size. As the cook proceeds the μ_{av} for a particular type of lignin sample increases, reflecting an increase in average charge density. The μ_{av}, measured at pH 12, decreases in the order Sd>Sb>Sr, except at the end of the cook, when the average charge densities of the Sb and Sr samples are similar. Associations between lignin and carbohydrate fragments are proposed to cause the lower mobility of black liquor compared to isolated dissolved lignin. Characterisations performed at pH 10 indicate that the isolated dissolved lignin samples may have a higher pK_a in the middle of the cook than the other samples.

INTRODUCTION

Lignin supports the cell wall and brings stiffness to the fibre in woody plants. About 27.7% of pine wood (*Pinus sylvestris*) consists of lignin, the other polymers being cellulose (40%) and hemicelluloses (24.9%).[2] The true structure of lignin is still unknown, but in principle, softwood lignin consists of hydroxymethoxyphenylpropane units linked together mainly by ether bonds but carbon-carbon bonds are present as well. This and the variety in type and amount of functional groups make lignin a heterogeneous branched polymer.

When wood is degraded in nature, lignin is partly depolymerized, oxidised and becomes a part of humic substances (HS) in soil.[3] During kraft pulping of wood, about 90 % of the lignin is removed and partially degraded lignin fragments are dissolved in the black liquor together with low molecular weight phenols originating from the lignin polymer. Besides inorganic components, black liquor also contains polymeric hemicellulose fragments and non-phenolic low molecular-weight products. The rate and extent of delignification are influenced by the accessibility and reactivity of the lignin[4, 5] as well as the hydrophilicity [4, 6] and size[7] of the lignin fragments. The main degrading reaction during pulping is aryl ether cleavage, where new phenol groups in the lignin are formed.[6, 8, 9] Since kraft pulping is carried out in highly alkaline conditions, the concentration of ionised groups is important for solubilising the lignin molecule. In addition, the size of the lignin fragments has to be small enough for the fragments to pass through the pores of the fibre to be removed from the fibre. In this context, the content of phenol groups and the size of lignin are reported, the latter commonly as molecular weight relative to polystyrene.

To obtain information about the distribution of ionised groups between lignin fragments of various sizes, the charge density of lignin samples can be estimated by capillary zone electrophoresis (CZE). In CZE, separation is achieved due to differences in mobility (μ), which is proportional to the charge-to-size ratio *i.e.* charge density of the sample components. If the shape of the solute is assumed

to be spherical, μ can be described by

$$\mu = q/ 6\pi\eta r \quad [1]$$

where q is the charge of the solute molecule, η is the viscosity of the separation buffer and r is the hydrodynamic radius of the sphere.[10] Although μ decreases with increasing molecular weight, the magnitude of the friction, *i.e.* the denominator in equation 1, depends on the geometry of the molecule. For spheres, the friction is proportional to the cubic root of the molecular weight (M), for random coiled polymers to the square root of the M.[11] A good correlation between mobility and surface area ($M^{-2/3}$) for proteins has also been reported.[12]

CZE has been used to characterise softwood lignin samples isolated from black liquor.[13] The mobility of underivatized lignin was obtained by the use of an alkaline buffer, *i.e.* the phenol groups of the lignin were dissociated during the electrophoresis. Electropherograms obtained by CZE have also been used for profile characterisation of HS.[14, 15, 16, 17] Due to the carboxylic acid groups in HS, the characterisations have mainly been performed at low pHs (3-5). To compare the charge densities of HS at different pHs, average electrophoretic mobilities have been calculated for HS samples from migration times[17] by approximating the distribution of the mobility to be Gaussian. Charge densities of different molecular mass fractions of humic acids (HA) have also been characterised and the fractions quantified by CZE.[18]

It is difficult to compare the mobilities of skewed distributions. Another problem connected with the interpretation of the mobility distribution is that sample components do not pass through the detection window at a constant velocity. This leads to a gradual increase in peak width as the residence time in the capillary increases. To compensate for this, the integrated peak area is commonly divided by the migration time when low molecular weight compounds are to be quantified.[19] In this report, a similar correction for the inconstant velocity is made and the average mobility (μ_{av}) of the distribution is determined

for lignin-containing samples. The procedure is used to compare the charge densities of black liquor, dissolved lignin (isolated from black liquor) and residual lignin (isolated from pulp), respectively, from various times of kraft cooking of softwood.

EXPERIMENTAL

Materials

All chemicals were of analytical grade. Two electrolytes were used in the capillary electrophoresis experiments: 100 mM glycine-sodium hydroxide pH 10.0 and 12.0, respectively They were prepared at room temperature by mixing appropriate volumes of deaerated 100 mM sodium hydroxide and 100 mM glycine. The final solutions were kept under nitrogen and used within five days.

Samples

Five flow-through kraft cooks of pine (*Pinus sylvestris*) were performed in order to obtain lignin-containing samples: black liquor (Sb), dissolved lignin (Sd) and residual lignin (Sr), see Table 1. Cooking conditions and isolation procedures for the dissolved and residual lignin samples are described in a previous paper.[20]

From one cook, six fractions of black liquor were collected and nitrogen was added to prevent oxidation. These samples were diluted by an equal volume of deionized water and characterised by capillary electrophoresis within 2 h. From another cook, similar fractions of black liquor were collected and the lignin dissolved in the black liquor was isolated by acid precipitation.

The Sd-samples correspond to 59% of the amount of lignin removed from the pulp. Three additional cooks were performed and interrupted after 90, 150 and 220 min. cooking time, to obtain pulps of different degrees of delignification.

TABLE 1
Sample Designation for Lignin-containing Samples from Flow-through Kraft Cooks of Pine, Corresponding Cooking Times and Kappa Numbers of pulps.

Cooking time (min)	Black liquor samples	Isolated lignin samples		Kappa number of pulps corresponding to		
		dissolved	residual	Sb	Sd	Sr
30-60	Sb 45	Sd 45				
60-90	Sb 75	Sd 75				
90-120	Sb 105	Sd 105				
120-150	Sb 135	Sd 135				
150-180	Sb 165	Sd 165				
180-210	Sb 195	Sd 195		19	22	
90			Sr 90			83
150			Sr 150			39
220			Sr 220			17

Residual lignin was isolated by acid dioxane extraction of these pulps. The efficiency of extraction was 13%, 50% and 53%, respectively. The isolated samples were dissolved in the pH 12-electrolyte over night (under N_2) and thereafter diluted by an equal volume of deionized water to a final concentration of 4.0 mg/L. All samples were filtered through a 0.45 μm filter prior to the CZE experiments.

Kappa Number and Klason Lignin

To estimate the amount of lignin, kappa numbers of the pulps were determined according to SCAN-C 1:77.[21] It should however be emphasised that the kappa number also reflects unsaturated structures other than lignin. The total

recovery of the dissolved lignin samples and the efficiency of the acid dioxane extraction were calculated from the obtained amount of lignin compared to the amount of acid-insoluble lignin (Klason lignin) of wood and pulps, respectively. Klason lignin was gravimetrically determined after swelling the pulp in 72% sulphuric acid at 30 °C for 1 h, followed by dilution to 3% and hydrolysis at 125 °C, 1.4 bar for 1 h.

Functional Group Analysis

Phenol group determinations of the isolated lignin samples were carried out by aminolysis according to Månsson.[22] This method also provided the acetylated samples for characterisation by size-exclusion chromatography (SEC). Carboxyl groups were determined by quantitative ^{1}H-NMR. Spectra of lignin (20-30 mg) in DMSO-d_6 (500 µL) were recorded on a Bruker 500 MHz spectrometer using a 45° pulse, 7507 Hz sweep width, 3 s delay, 2 s acquisition time and a time domain of 29k. 512 scans were recorded, and the spectrum was processed without any zero-filling or window function. Trimethylsilyl propionic acid 2,2,3,3-d_4 (TSP) was used as internal shift and quantitative standard. The spectra were analyzed as described in the literature.[23, 24] The RSD of the carboxyl group determination was 10% and the minimum detectable concentration (MDC) was 0.1 mmol/g.

Size-Exclusion Chromatography

Acetylated lignin (40 µg) dissolved in tetrahydrofuran (THF) was characterised at room temperature using THF as the mobile phase. The SEC system consisted of a Rheodyne 7125 injector, a set of three columns connected in series, 10^4Å, 500Å, 100Å (ultrastyragel, Waters), and a Waters 510 pump (1

mL/min). The solutes were detected by a Waters 410 refractive index detector. Polystyrene (PS) standards in the range 580 D to 350 kD (Polymer Laboratories Ltd., UK) were used to calibrate the SEC-system. Data acquisition and calculations were carried out using Baseline software (Waters).

Capillary Zone Electrophoresis

CZE was performed on an Applied Biosystem 270A-HT instrument with a 72 cm x 50 μm I.D. fused silica capillary. The sample vials were filled with 50 μL of each sample solution and 5 μL of n-C_{14} was added to prevent oxidation. To minimise evaporation, the sample compartment was thermostated at 7 °C. The samples were introduced into the capillary by vacuum for 1.5 s preceded by 0.5 s of pyridine (0.1% v/v) as neutral marker. A voltage of 20 kV was applied and the temperature of the separation compartment was kept at 30 °C. After each run the capillary was conditioned by applying 30 kV for 2 min, flushing with 0.1 M sodium hydroxide for 2 min and with electrolyte for 3 min. Detection was carried out at 254 nm, 50 cm from the injection site. Electropherograms were registered and calculations were performed using ELDSPRO Labdata system (Chromatography Data Systems AB, Kungshög, Sweden).

RESULTS AND DISCUSSION

Isolated Lignin Samples

In accordance with equation 1, the amounts of phenol and carboxyl groups as well as fragment size, will influence the mobility of kraft lignin samples. The concentrations of phenol and carboxyl groups in the isolated dissolved lignin (Sd)

TABLE 2
Concentration of Phenol and Carboxyl Groups and Number-Average Molecular Weight (M_n) of Isolated Lignin Samples from Kraft Cooking of Pine. Sample Designations According to Table 1.

Isolated lignin samples		Phenol groups	Carboxyl groups	M_n[1]
		mmol/g	mmol/g	(relative PS)
Dissolved lignin	Sd 45	2.4	n.d.[2]	790
	Sd 75	2.6	0.46	980
	Sd 105	2.7	0.47	1300
	Sd 135	2.7	0.37	1500
	Sd 165	2.8	0.71	1600
	Sd 195	3.0	<0.10	1700
Residual lignin	Sr 90	2.2	<0.10	3400
	Sr 150	2.7	<0.10	4600
	Sr 220	3.2	0.18	3500

[1] acetylated samples. [2] = not determined.

and residual lignin (Sr) samples corresponding to different cooking times are shown in Table 2. Due to the cleavage of β-arylether linkages, the amount of phenol groups increases as the cook proceeds in both types of lignin samples. Because of the non-oxidative conditions during kraft cooking, the amount of carboxyl groups in respective lignin sample is considerably lower compared to the amount of phenol groups. The concentration of carboxyl groups in the analysed Sd samples is about constant until a maximum at around 165 minutes cooking time. At the end of the cook, however, the content of carboxyl groups decreases and is below the MDC of the method. The concentration of carboxyl groups in the Sr samples is below the MDC until the end of the cook, when the concentration is higher than for the corresponding Sd sample.

The influence of size on mobility depends on the molecular weight (M) and conformation of the lignin, *i.e.* the friction it exerts against movement. The

number-average molecular weight (M_n) of acetylated samples is reported in Table 2. To compare the influence of size on mobility, similar conformations of the samples must be assumed, which may not be true for the samples in this study, considering their different origins and isolation methods. The M_n of the Sd samples increases until the end of the cook where it levels out. All of the Sr samples have a higher M_n compared to the Sd samples. The non-uniform change of M_n with respect to cooking time of the residual lignin may be due to the low efficiency (13%) of the acid dioxane extraction of the pulp from the beginning of the cook. At this stage of delignification the pulp is not completely defibrated, and it is probable that only easily accessible lignin fragments of fairly low molecular size are removed. It should be noted that this isolation method has not been applied to softwood kraft pulps with high lignin content, such as the Sr 90-pulp, before. The efficiency of isolation of the other residual lignin samples was about 50% and the M_n for these samples indicates a decrease in molecular weight from the middle to the end of the cook.

Correction for Peak Broadening

Lignin samples have a broad mobility distribution range. Due to the decrease in velocity, the peak width increases as the migration time increases. To compensate for this, the detector signal was recalculated to give a corrected signal (z_i) using the equation

$$z_i = B_i + h_i \times t_k/t_i \qquad [2]$$

where B_i, h_i and t_i are the baseline signal, the peak height and the migration time, respectively, of each datapoint i of the distribution; t_k is a constant equal to the migration time of the peak maximum of the integrated distribution and is used to obtain a peak maximum of about the same intensity as the original

distribution. Electropherograms before and after time correction are shown in Figure 1 for one of the isolated lignin samples. The importance of time correction for a particular sample increases the more the distribution deviates from Gaussian shape and the broader the distribution range is.

Mobility Distributions of Lignin-Containing Samples

The profiles of the mobility distribution are different for different types of lignin-containing samples. Time-corrected electropherograms for some samples, residual lignin (Sr), dissolved lignin (Sd) and black liquor (Sb), characterised at pH 12, are shown in Figures 2, 3 and 4, respectively. The samples correspond to the beginning, middle and end of a kraft cook. Because of their negative charge, lignin samples have longer migration times than the neutral marker (NM). The mobility of lignin is directed towards the anode but it is swept to the cathode by the electroendosmotic flow (EOF). Thus the migration time increases with increasing mobility of the lignin.

Residual lignin samples have a smooth, Gaussian-shaped distribution (Figure 2). It should be noted that the mobility distributions, *i.e.* charge densities, increase during the cook, although the sizes of the residual lignin samples are non-uniform (Table 2). The absorbtivity of lignins at a given wavelength depends on the nature of the substituents on the aromatic ring.[25] The absorbtivity of the Sr 90 sample is lower than for the other Sr samples, indicating differences in the composition of functional groups. The dissolved lignin samples isolated from black liquor collected in the beginning (Sd 75) and middle (Sd 135) of the cook have some peaks superimposed on their distributions, Figure 3. The Sd 75 sample contains larger amounts of low mobility components, *i.e.* has a slightly skewed distribution, whereas the other Sd samples are fairly Gaussian in shape. All of the black liquor samples have rough distributions, which deviate from Gaussian shape, probably because of the presence of low-mobility components, Figure 4.

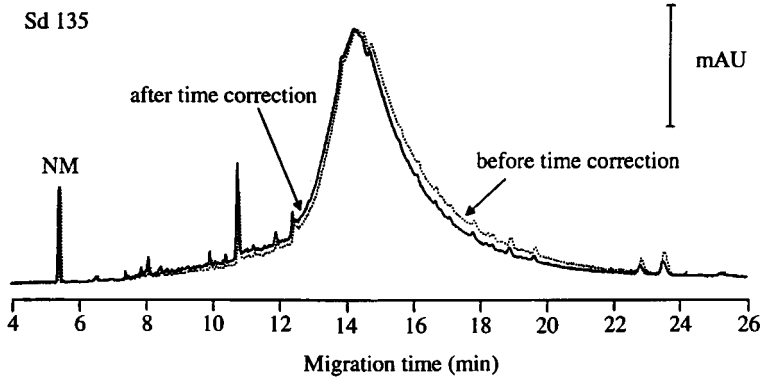

FIGURE 1. Electropherogram of a lignin sample before and after time correction according to equation 2. Capillary, 0.72 m x 50 μm I.D.; applied voltage 20 kV; electrolyte, 0.1 M glycine-NaOH, pH 12; temperature 30 °C; detection 254 nm.

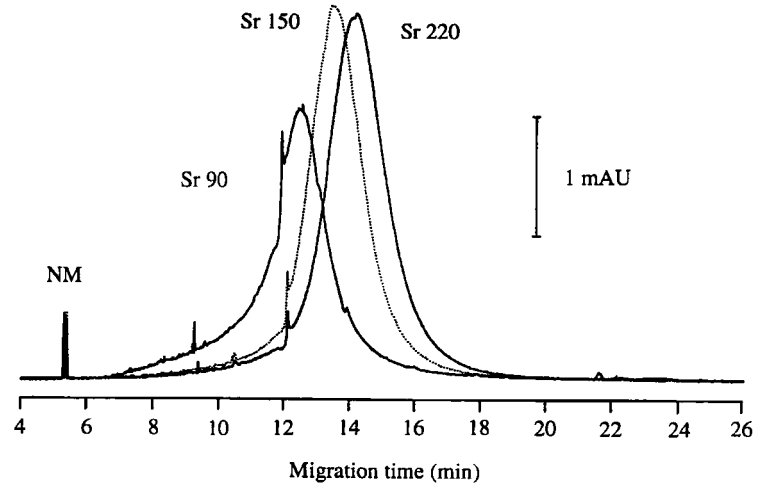

FIGURE 2. Corrected electropherograms of residual lignin isolated from softwood kraft pulp. The cooks were interrupted in the beginning (Sr 90), in the middle (Sr 150) and at the end (Sr 220) of a kraft cook of softwood. Conditions as in Figure 1.

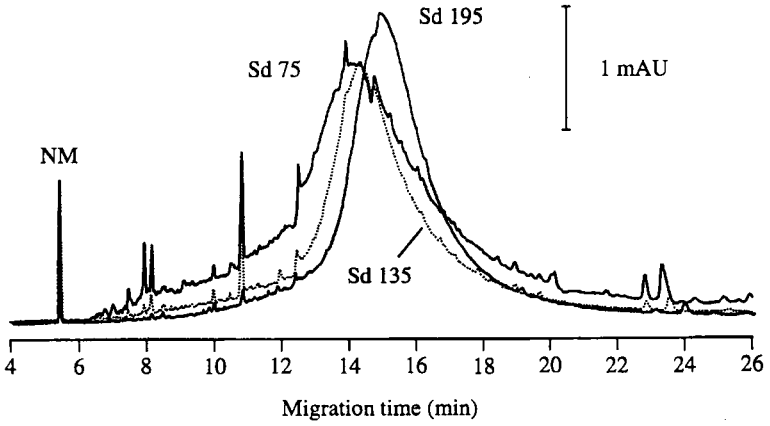

FIGURE 3. Corrected electropherograms of dissolved lignin isolated from black liquor collected in the beginning (Sd 75), in the middle (Sd 135) and at the end (Sd 195) of a kraft cook of softwood. Conditions as in Figure 1.

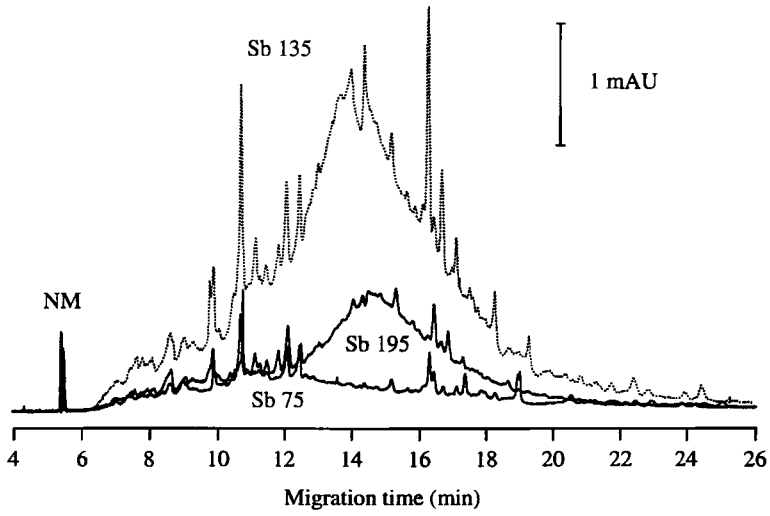

FIGURE 4. Corrected electropherograms of black liquor collected in the beginning (Sb 75), in the middle (Sb 135) and at the end (Sb 195) of a kraft cook of softwood. Conditions as in Figure 1.

The main portion of the lignin is removed from the wood during the bulk delignification phase, roughly corresponding to samples collected between 60 and 180 min. The differences in detector response, Figure 4, thus mainly reflect different concentrations of lignin in black liquor since these samples were diluted in the same way prior to characterisation.

Due to the differences in profiles and absorbance in the electropherograms it is difficult to compare the mobility between the different types of lignin samples. To ease comparison, the average mobility (μ_{av}) was determined after calculation of the centre of gravity of the distributions (t_g).

Calculation of the Average Mobility

The centre of gravity (t_g) of the corrected mobility distributions was determined according to

$$t_g = \int_{t_0}^{t_f} t_i f(t)dt \Big/ \int_{t_0}^{t_f} f(t)dt \qquad [3]$$

where t_0 is the initial and t_f is the final migration time of the distribution, t_i the time and $f(t)$ the corrected detector signal as a function of time. The average mobility (μ_{av}) of the distribution was calculated using the common equation that relates the migration time with the electrophoretic mobility. Due to ionisation of the silanolic groups, the migration time of a solute is also influenced by the EOF, which is caused by the movement of hydrated cations adsorbed onto the glass surface. In this study, the migration time of pyridine was used to determine the magnitude of the EOF. The average mobility (μ_{av}) of the distribution was calculated by replacing the migration time of a peak (t_r), used in the common equation, with t_g:

$$\mu_{av} = \ell\, L/U\, (1/t_g - 1/t_{NM}) \qquad [4]$$

where ℓ is the length to detector, L total length of the capillary, U applied voltage and t_{NM} the migration time of pyridine. Lignin samples only contain anions and thus the μ_{av} will be negative. Since this study only concerns the magnitude of the mobility, the $|\mu_{av}|$ is reported.

The Average Mobility of Lignin-Containing Samples

The μ_{av} of black liquor, dissolved lignin and residual lignin samples characterised at pH 12 are shown in Figure 5. The 95% confidence limits of the mean were calculated from triplicates. The data points of black liquor and dissolved lignin samples are plotted at the mean collection time for each range, Table 1. For all samples, the charge density increases as the cook proceeds. The dissolved lignin samples have the highest mobility at each time throughout the cook. The difference between dissolved lignin and residual lignin is significant, reflecting the higher charge density of the former. This is also in agreement with the difference in M_n between the two types of lignin samples. The lower concentration of charged groups in the Sr 90 and Sr 150 samples, compared to the Sd samples, also contributes to lowering the μ_{av} of these samples. In spite of the higher concentration of charged groups in the Sr 220, the dissolved lignin still has a higher μ_{av} because of its smaller size. The residual lignin samples from the beginning and from the end of the cook have significantly different μ_{av}. This can be explained by different concentrations of ionised groups, since these samples have similar M_n. Besides lignin, black liquor also contains low molecular-weight molecules and carbohydrate polymer fragments. According to equation 1, an increase in size decreases the mobility of a particular solute. The lower mobility of black liquor compared to isolated dissolved lignin samples may be due to associations between lignin and carbohydrate fragments. According to these results, the residual lignin has a considerably lower charge density than the lignin

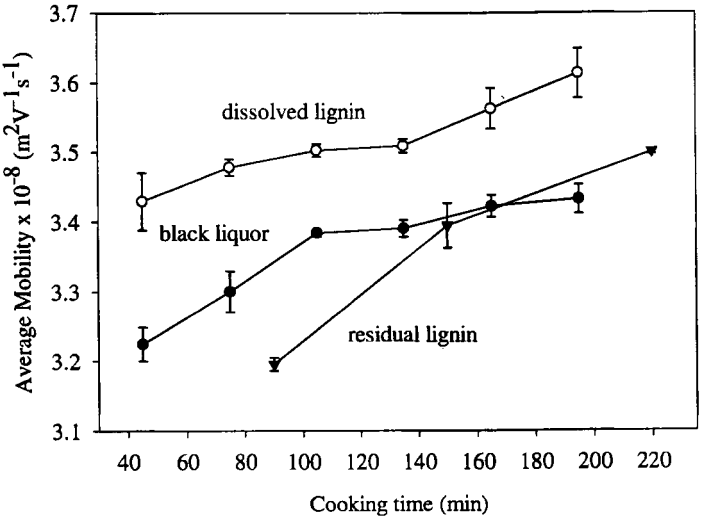

FIGURE 5. Average mobility (μ_{av}) at pH 12 of black liquor and isolated lignin samples corresponding to different cooking times. μ_{av} calculated according to equation 4.

removed from the pulp. The introduction of either phenol or carboxylic groups into pulp lignin would ease the extraction of lignin.

The Influence of pH on Mobility

To study the effect of the dissociation of phenol groups on the mobility, the samples were also characterised at pH 10. The phenol groups in lignin do not have a single pK_a-value. According to literature half of the phenol groups in soda and kraft lignin are dissociated in the pH-range 9.2 - 11.5.[26, 27, 28] Lignin-related phenol groups also differ in acidity over a wide range (pK_a 6.2 – 11.3) depending on substitution.[29] The average pK_a of the phenol groups of the isolated lignin samples in this study may thus be estimated to be around 10. If this assumption is

accepted, an extensive dissociation of the phenol groups can be expected at pH 12 whereas at pH 10 the degree of dissociation should be considerably lower. Keeping in mind that the characterisation in this study was performed at 30°C and that the ionic strength is higher at pH 12 than at pH 10 in the electrolyte used, the shift in μ_{av} only reflects relative differences in pK_a. If the phenol groups in the studied samples have the same pK_a-value the shape of the μ_{av} graph should be retained.

As expected, the μ_{av} of all samples is lower at pH 10 than at pH 12 (Figure 6 *cf.* 5). However, the decrease in average mobility is not equal for the three series when the pH is lowered. For residual lignin and black liquor samples, the change in μ_{av} during the cook is about the same irrespective the degree of dissociation and thus the shape of the μ_{av}-graph is similar at the two pH levels studied. In contrast, the μ_{av}-graph of the isolated dissolved lignin characterised at pH 10 (Figure 6) differs from that characterised at pH 12 (Figure 5). The broad confidence interval of the dissolved lignin sample corresponding to the beginning of the cook makes it difficult to draw any certain conclusion concerning the relative acidity of this sample. The main difference between the μ_{av}-profiles at the studied pHs, is caused by a larger decrease in mobility at pH 10 for the dissolved lignin samples that correspond to the middle of the cook. This may indicate a higher pK_a-value for the phenol groups in the lignin fragments dissolved during the bulk delignification phase, compared both to the other dissolved lignin samples and to the black liquor and residual lignin samples. To determine the pK_a of lignin, it is however important to keep the ionic strength constant and to measure the mobility over a wider pH range.

The electropherograms obtained at pH 10 of the samples corresponding to the end of the cook are shown in Figure 7. The figure illustrates the importance of determining the centre of the mobility distribution. The electropherogram of the black liquor (Sb) appears to have a shorter average migration time, *i.e.* lower charge density, than the residual lignin (Sr) sample. By calculating the centre of the distribution and determining the μ_{av}, the opposite relation is revealed, *cf.* Figure 6.

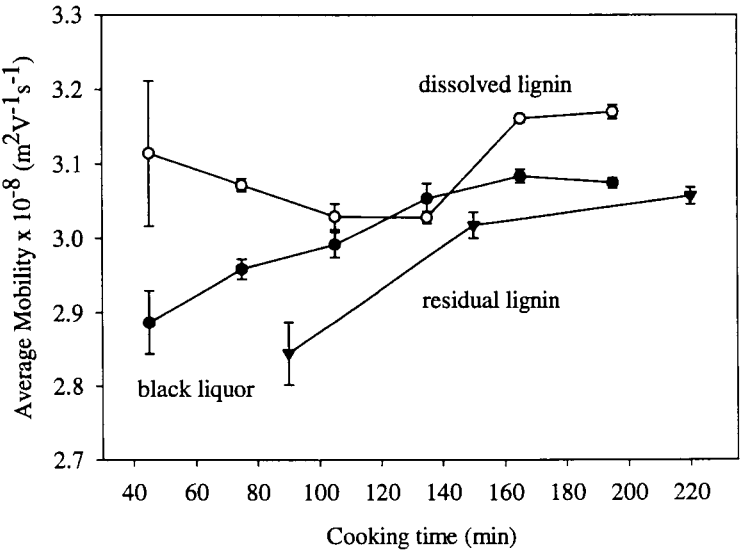

FIGURE 6. Average mobility (μ_{av}) at pH 10 of black liquor and isolated lignin samples corresponding to different cooking times. μ_{av} calculated according to equation 4.

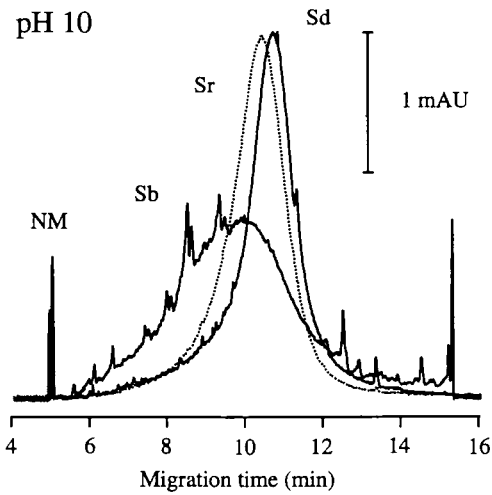

FIGURE 7. Corrected electropherograms of black liquor (Sb 195), isolated dissolved lignin (Sd 195) and isolated residual lignin (Sr 220). The samples were characterised at pH 10, other conditions as in Figure 1.

CONCLUSIONS

Black liquor (Sb), dissolved lignin (Sd) and residual lignin (Sr) samples obtained from kraft cooking of softwood, have different mobility distribution profiles as determined by CZE. By calculating the average mobility μ_{av} of time-corrected electropherograms, it is possible to compare the changes in average charge density of lignin samples during a cook.

As the cook proceeds the μ_{av} for a particular type of lignin sample increases, reflecting an increase in average charge density. The μ_{av}, measured at pH 12, decreases in the order Sd>Sb>Sr, except at the end of the cook, when the average charge densities of the Sb and Sr samples are similar. Associations between lignin and carbohydrate fragments may be the reason for the lower mobility of black liquor lignin compared to isolated dissolved lignin. The change in μ_{av} when the pH is lowered from 12 to 10 indicates differences in acidity between the dissolved lignin samples corresponding to the middle of the cook and the rest of the samples.

Due to the comprehensive dissociation at pH 12, this electrolyte is better to use than the pH 10 electrolyte when estimating the relative charge density of kraft lignin samples by CZE.

REFERENCES

1. Present address: Stora Enso Research, Box 9090, SE-650 09 Karlstad, Sweden

2. E. Sjöström, in Wood Chemistry Fundamentals and Applications, Chap. 7, Academic Press, Inc., Orlando, 1993.

3. S.M. Schevenko and G.W. Bailey, Crit. Rev. Environ. Sci. Technol., 26(2), 95 (1996).

4. J. Gierer, Wood Sci. Technol., 14, 241 (1980).

5. P. Whiting and D.A.I. Goring, J. Wood Chem. Technol., 1(2), 111 (1981).

6. S. Ljunggren, Svensk Papperstidn., 83(13), 363 (1980).

7. P.A. Ahlgren, W.Q. Yean and D.A.I. Goring, Tappi, 54(5), 737 (1971).

8. J. Gierer, Svensk Papperstidn., 73(18), 571 (1970).

9. G. Gellerstedt, E.-L. Lindfors, C. LaPierre and B. Monties, Svensk Papperstidn., 87(9) R61 (1984).

10. S.F.Y. Li, in Capillary Electrophoresis. Principles, practice and applications. Chap. 5, Elsevier Science Publishers B.V., Amsterdam, Netherlands, 1992.

11. J.C. Giddings, in Unified Separation Science, pp. 77-79, John Wiley & Sons, Inc., New York, 1991.

12. E.C. Rickard, M.M. Strohl and R.G. Nielsen, Anal. Biochem., 197, 197 (1991).

13. E. Sjöholm, N.-O. Nilvebrant and A. Colmsjö, J. Wood Chem. Technol. 13(4), 529 (1993).

14. A. Rigol, J.F. López-Sánchez and G. Rauret, J. Chromatogr., A 664, 301 (1994).

15. D. Fetsch and J. Havel, J. Chromatogr., A 802, 189 (1998).

16. P. Schmitt-Kopplin, N. Hertkorn, H.-R. Schulten and A. Kettrup, Environ. Sci. Technol., 32, 2531 (1998).

17. Ph. Schmitt-Kopplin, A.W. Garrison, E.M. Perdue, D. Freitag and A. Kettrup, J. Chromatogr., 807, 101 (1998).

18. A. Rigol, M. Vidal and G. Rauret, J. Chromatogr., A 807, 275 (1998).

19. K. Kitagishi, in Handbook of capillary electrophoresis applications, Chap. 2, H. Shintani and J. Polonský (eds.), Chapman & Hall, London, 1997.

20. E. Sjöholm, K. Gustafsson, E. Norman, A. Colmsjö, J. Liq. Chromatogr. Relat. Technol., (1999) in press.

21. SCAN-C 1:77, Secretariat, Scandinavian Pulp, Paper and Board Testing Committee, Box 5604 SE-114 86 Sockholm, Sweden.

22. P. Månsson, Holzforschung, 37, 143 (1983).

23. S. Li and K. Lundquist, Nordic Pulp Paper Res. J., 3, 191 (1994).

24. P. Froass, A.J. Ragauskas and J.-er. Jiang, J. Wood Chem. Technol., 16(4), 347 (1996).

25. S.Y. Lin, in Methods in lignin chemistry, p. 223, S.Y. Lin and C.W. Dence (eds.), Springer-Verlag Berlin, Germany, 1992.

26. J.J. Lindberg, Finska Kemists. Medd. 68(1), 5 (1959).

27. G.B. Shtreis and V.M. Nikitin, Zh. Prikl. Khim., 40(8), 1814 (1967) (*in Russian*).

28. D.L. Woerner and J.L. McCarthy, in Proceedings of the 4th International Symposium on Wood and Pulping Chemistry, Paris, France, 27-30 April 1987, Vol. 2, p.71.

29. M. Ragnar, C.T. Lindgren and N.-O. Nilvebrant, in Proceedings of the 10th International Symposium on Wood and Pulping Chemistry, Yokohama, Japan, 7-10 June 1999, Vol. 2, p. 154.