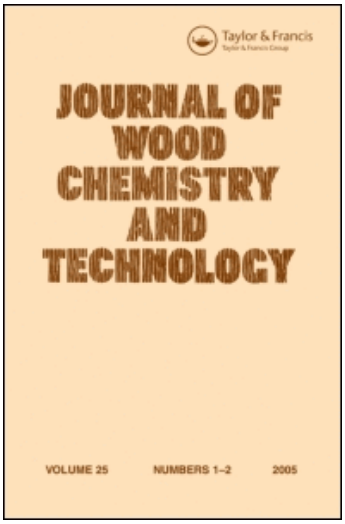

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

Characterization of Dissolved Kraft Lignin by Capillary Zone Electrophoresis

Elisabeth Sjöholm[a]; Nils-Olof Nilvebrant[a]; Anders Colmsjö[b]
[a] STFI, Swedish Pulp and Paper Research Institute, Stockholm, Sweden [b] National Institute of Occupational Health, Solna, Sweden

To cite this Article Sjöholm, Elisabeth , Nilvebrant, Nils-Olof and Colmsjö, Anders(1993) 'Characterization of Dissolved Kraft Lignin by Capillary Zone Electrophoresis', Journal of Wood Chemistry and Technology, 13: 4, 529 — 544
To link to this Article: DOI: 10.1080/02773819308020532
URL: http://dx.doi.org/10.1080/02773819308020532

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 13(4), 529-544 (1993)

CHARACTERIZATION OF DISSOLVED KRAFT LIGNIN BY CAPILLARY ZONE ELECTROPHORESIS [1]

Elisabeth Sjöholm and Nils-Olof Nilvebrant
STFI, Swedish Pulp and Paper Research Institute
Box 5604, S-114 86 Stockholm, Sweden
and
Anders Colmsjö
National Institute of Occupational Health
S-171 84 Solna, Sweden

ABSTRACT

This report presents a method to characterize underivatized lignin by Capillary Zone Electrophoresis (CZE), in which separation is achieved by differences in mobility due to differences in the charge-to-size ratios of the solutes.

A comparison has been made between the separation of lignin by CZE and earlier studies[1] regarding the size and phenolic content of dissolved lignin. The samples were isolated at different times from a flow-through kraft cook and characterized by CZE in an alkaline solution. The resulting electropherograms show a molecular mobility distribution. The molecular mobility distribution of each fraction is in good agreement with its molecular size distribution and phenolic content. As the cook proceeds, the mobility of the lignin fragments increases with increasing phenolic content. Although the molecular size distribution shows a slight increase

[1] A preliminary report was presented at the EUCHEM conference on Capillary Electroseparations, 1991, Storlien, Sweden.

in molecular weight at the end of the cook according to Size Exclusion Chromatography (SEC), this does not seem to affect the mobility distribution.

The change in mobility distribution may be a useful new parameter to be used in lignin characterization, as the dissolution of lignin is influenced by the number of ionized groups and by the size of the lignin fragments.

Keywords: alkali lignins, analysis, black liquor, capillary electrophoresis, gel permeation chromatography, mobility distribution, size distribution, phenol groups.

INTRODUCTION

Lignins are polymers consisting of phenylpropane units covalently linked to form a branched heterogenic structure. The molecular size and number of functional groups are important factors influencing the dissolution of lignin in the pulping process. To understand how pulping may be optimized, it is important to be able to analyse and interpret the nature of both the dissolved and the pulp lignin. Isolated lignin preparations have been characterized according to i.e. their elemental composition, functional groups (phenolic, carboxylic and methoxylic)[1] and degree of crosslinking[2].

The molecular weight distribution of lignins has been extensively studied by SEC[3-5]. Aqueous alkaline SEC has also been performed[5,6]. A disadvantage of these methods is that the fractionation may be disturbed by adsorption. Phenols are the main functional group that contributes to lignin solubility in alkali. Analysis in an aqueous medium therefore requires a pH value above the pK_a values of the phenols in order to avoid precipitation in the column. Since there is a general lack of packing materials that are stable in strong alkaline solutions and to avoid adsorption, the conventional way to analyse kraft lignin by SEC is to use polystyrene gels and derivatized samples and to elute with tetrahydrofuran. The obvious drawback is that both the size and the

molecular weight of the lignin are changed when the phenolic groups are blocked by derivatization[7]. This report describes the use of CZE for the characterization of dissolved kraft lignin in strong alkaline environments without derivatizing the sample.

Capillary Electrophoresis

Capillary electrophoresis is an analytical method, which has been widely used to analyse polar and medium polar compounds of varying sizes in the biomedical field. Generally, separation is achieved by the migration of charged solutes under the influence of an applied electrical field. The supporting medium in traditional electrophoresis suppresses convection and limits the voltage. It can also cause undesirable interactions between the solute and the medium. Fundamental work showed the advantage of performing electrophoresis in a free solution using a quartz capillary tube[8], which was later developed to CZE[9,10] by decreasing the inner diameter of the tube.

CZE is now accepted as an analytical technique in many areas outside the original applications e.g. chemical analyses of sulphonic acids[11], low molecular weight carboxylic acids[12-14] , chlorophenols[15], carbohydrates[16] and inorganic ions[13].

CZE is today characterized by a high efficiency. It can be used for the analysis of samples of both low and high molecular weight, consisting of both neutral and charged solutes. Small sample volumes are sufficient as well as a minimum of sample pretreatment. Because of the small sample volumes and narrow capillaries, there are limitations in the sensitivity with respect to concentration, although the sensitivity is comparable to HPLC.

Theory of Capillary Zone Electrophoresis

The time, t, for a solute to migrate from the injector to the detector is given by

$$t = l L / \mu V \qquad [1]$$

where l is the distance between the capillary end where the injection is made and the detector, L is the entire length of the capillary, μ is the electrophoretic mobility of the solute and V is the applied voltage. There is also an electro(endo)osmotic flow (EOF) affecting the apparent migration time of a solute, caused by the negative charges of the silanolic groups on the glass surface. The flow profile is flat[17]. It contributes equally to all components regardless of their position and does not cause any considerable zone spreading as does a laminar flow profile. The observed migration time t_{obs} is:

$$t_{obs} = l\,L / (\mu + \mu_{eo})\,V \qquad [2]$$

where μ_{eo} is the electroosmotic mobility. The mobility μ is of opposite sign to μ_{eo} when anions are considered. The EOF results in a net flow of liquid towards the cathodic end of the tube (see the arrows in Figure 5). An increase in the charge-to-size ratio of an anion leads to a longer observed migration time as will be shown below.

The magnitude of the EOF is highly dependent on the pH[17,18]. This can be measured by adding a neutral molecule which will coelute with the EOF. Due to ionization of the silanolic groups at the glass surface, the EOF increases with increasing pH up to pH 10, where the effect becomes stable. The condition of the capillary is also vitally important for the resulting mobility[19].

Besides the applied voltage and the EOF, other factors that influence migration time and separation are the buffer composition and concentration[20-24], the cation used[25], the temperature[26] and the addition of modifiers[27,28]. Separation by CZE can thus be optimized by adjusting these factors.

The expression for efficiency, calculated as the number of plates, is derived from chromatography[29]. Taking the EOF into account, the efficiency is given by

$$N = (\mu + \mu_{eo})\,V / 2D \qquad [3]$$

where D is the molecular diffusion coefficient of the analyte. An increase in temperature causes band broadening due to an increase in molecular diffusion and thus a decrease in N. This expression, indicating a linear relationship between efficiency and applied voltage, is valid only in the absence of Joule heating[20]. In other words, N passes through a maximum with respect to voltage. As long as the ionic strength of the electrolyte is kept fairly low, Ohm's law is valid indicating that the heat generated is dissipated[22,26]. Overloading may lead to an asymmetric peak shape[23] and may also contribute

TABLE 1

Phenolic contents of the studied fractions and corresponding cooking times and temperatures when the black liquors were withdrawn[1].

Fraction	Phenolic groups/ C_9-unit	Cooking temperature (° C)	Cooking time (min)
2	0.62	160	170
4	0.66	170	150
6	0.71	170	210

negatively to the efficiency[11]. For convenience, the expression for the efficiency is calculated according to:

$$N = 5.54 (t_{obs} / w_{1/2})^2 \qquad [4]$$

where $w_{1/2}$ is the peak width at half height.

RESULTS AND DISCUSSION

A series of dissolved kraft lignins isolated from a flow-through kraft cook, characterized by Robert et al.[1] were analysed. Dissolved lignins were withdrawn on six different occasions. The lignins were isolated by acid precipitation and extracted with n-pentane to remove elemental sulphur and extractives and in dioxane-water (9:1) to purify the lignin from any coprecipitated carbohydrates[30]. The samples were then freeze-dried and stored at room temperature.

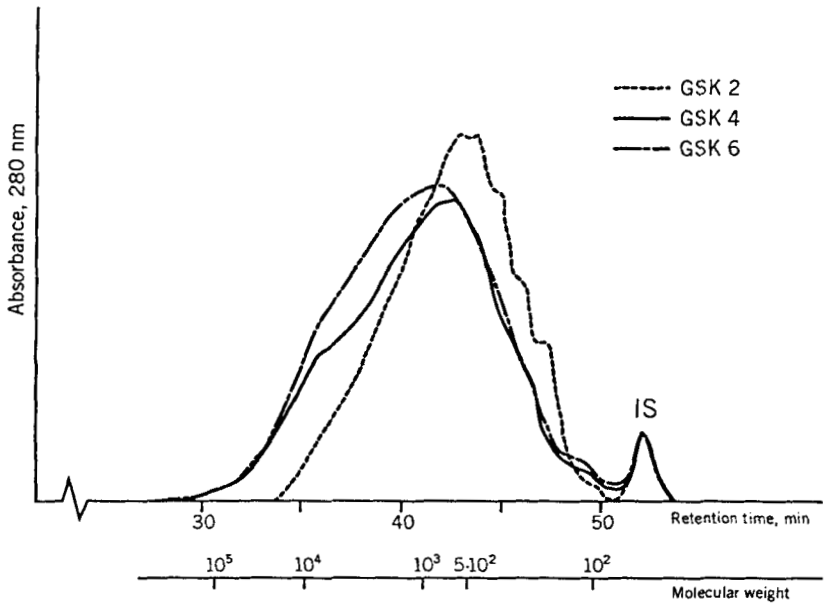

FIGURE 1. Size exclusion chromatograms of acetylated lignins obtained from a flow-through kraft cook[1]. Molecular weight according to polystyrene standards, I.S. = acetone. The lignin samples were detected by UV-absorption at 280 nm.

Table 1 show data from three of the fractions, corresponding to the initial stage (fraction 2) and to the beginning and end of the bulk phase (fractions 4 and 6). The amounts of free phenolic groups were determined by aminolysis[1]. The frequency of phenolic groups per phenylpropane units increases with increasing cooking time.

Figure 1 shows size exclusion chromatograms of isolated lignins, derivatized by acetylation of the phenolic groups. The average molecular size of the lignin increased slightly as the cook proceeds. A wider molecular size distribution was seen in the bulk phase compared to the initial material.

The electropherograms in Figure 2 shows the mobility of the lignins at pH 10.0. As the cook proceeds, the mobility of the dissolved lignin

increases. Because of the negative charge of the sample, the mobility is directed towards the anode. Consequently, the lignin passes the detector later with increasing charge density (see equation [2] ff.). Even though the molecular size increases during the cook, the greater number of phenolic groups gradually leads to a change in the mobility of the macromolecules. The increase in the charge density is probably due to the phenolic hydroxyl groups formed when α- and β-ether bonds in the kraft lignin are cleaved during the cook. The cleavage of methyl aryl ether bonds may also contribute to the formation of phenolic groups. In the dissolved kraft lignin there are also carboxylic acid groups present[1]. It is not known if these groups are solely native or formed during the cook. As the conditions in the kraft cook are non-oxidative, the formation of additional carboxylic acid is not likely to occur[31]. It seems likely that the amounts of carboxylic acid groups present in the dissolved kraft lignin do not vary significantly throughout the cook. Therefore, these groups contribute equally to the mobility of the different fractions shown in Figure 2. Thus, the main contribution to the increase in the mobility is probably due to the ionized phenolic hydroxyl groups formed from the cleavage of the above mentioned ether linkages.

Runs were also made with a 10 % (v/v) addition of acetonitrile in order to exclude adsorption effects of the capillary wall. This increased the migration time, but did not influence the separation. Since lignin is a negatively charged polyelectrolyte, there is no risk of electrostatic attraction to the capillary wall, because the silica capillary presents a negatively charged surface in alkaline solutions. Band broadening due to adsorption or attraction to the silica capillary wall can thus be excluded.

When the voltage was increased, the migration time of the lignin samples decreased. This gave a more concentrated profile with sharper peaks (in accordance with equation [3]), but yielded no additional information.

To examine if high molecular weight compounds with multiple charges can be analysed with CZE with high efficiency, we analysed polystyrene sulphonates (PSS). These standards are frequently used for

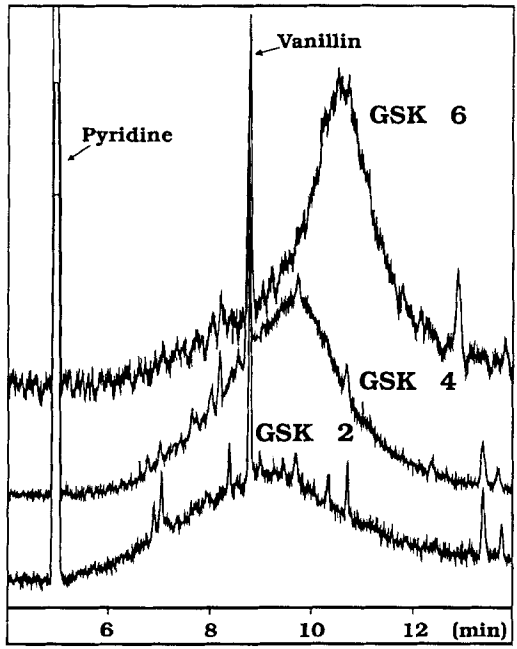

FIGURE 2. Electropherogram of underivatized dissolved lignin, obtained from a flow-through kraft cook and detected at 254 nm. The lignin samples were isolated from the initial phase, GSK 2 and from the initial and final stage of the bulk phase, GSK 4 respective GSK 6. Pyridine was added to each sample as a neutral marker. Vanillin was identified in all of the fractions. Electrophoresis was performed in a 72 cm x 50 μm I.D. uncoated capillary at 20 kV using a 0.1 M glycine buffer, pH 10.0.

calibration in aqueous SEC. In spite of their different molecular weights (35·000, 100·000 and 200·000 Daltons) the observed migration times were the same. In addition, these electropherograms show a relatively narrow peak (Figure 3) since the PSS has sulphonic acid groups evenly distributed along the polymer. Consequently the high molecular weight of lignin is not a limitation in CZE.

The shapes of the electropherograms of the lignin samples are similar to those of size exclusion chromatograms of lignins in general. In SEC, the distribution is related to molecular size and it is likely that the

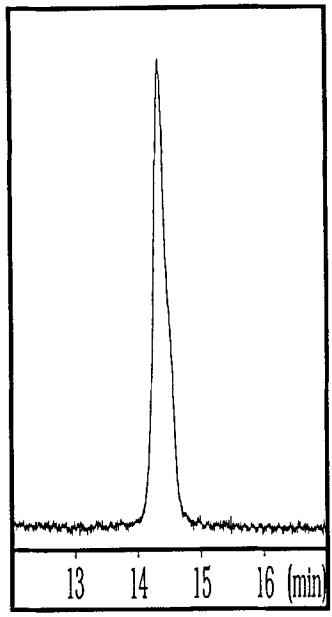

FIGURE 3. Electropherogram of a sulphonated polystyrene standard, M_p = 200·000 (M_w/M_n = 1.1). Conditions as in fig. 2. UV-absorption at 230 nm.

electropherograms also exhibits a distribution; a distribution of the molecular mobility.

According to this interpretation, the dissolved kraft lignins consist of a wide distribution of fragments with different amounts of negatively charged groups. It is apparent that CZE does provide information concerning the charge distribution within a sample of dissolved kraft lignin. Comparing the different fractions in Figure 2, the change in the charge distribution is obvious. The charge distribution is rather wide in the early stage of the cook (sample GSK 2) and becomes narrower at latter stages (sample GSK 6). Since the evenly distributed charged groups of the polystyrene polymers give rise to a narrow mobility distribution, the lignin fragments of sample GSK 6 may be more uniform with respect to

phenolic groups per lignin polymer chain than in the earlier stage. The wide distribution of the lignin fragments in sample GSK 2, may be due to a more non-uniform charge distribution within the lignin macromolecule. Intermolecular interactions between the fragments may to some extent contribute to the shape of the electropherograms, as in the case of SEC. However, under the alkaline conditions used in this CZE-study, these misleading effects are minimized.

In addition to the information gained by detection at 254 nm, higher wavelengths were used in order to determine the absorptivity originating from e.g. conjugated double bonds or conjugated carbonyl structures. However no major spectral changes were recorded between the fractions. This means that the proportion of chromophoric structures in the dissolved lignin determined by capillary electrophoresis do not appreciable change during the cook.

The main peak superimposed upon the polymeric material was identified as vanillin. This identification was based on the electroosmotic mobility and spectral pattern at different pH´s was compared with data for a vanillin standard. Vanillin is readily formed from lignin by autoxidation in alkaline media. It has probably been formed during the storage or sample preparation.

Reproducibility, linearity and sensitivity expressed as minimum detectable concentration (MDC) were determined for vanillin under the same conditions as those used for the analysis of lignin samples. Ten consecutive runs were made with a vanillin standard (10 μg/ml). The reproducibility, measured as the relative standard deviation, was 0.2 % for the retention time and 1.8 % for the area. The concentration was linear related to the area in the explored range between 0.5 and 100 μg/ ml. The MDC was established to be 0.5 μg/ ml. The efficiency for vanillin in fraction 2, measured as the number of plates according to equation [3], was calculated to be 207·000, which is a very high number, compared to other analytical techniques based on liquid chromatography.

A lignin sample (GSK 4) was analysed at both pH 10.0 and pH 12.0, Figure 4. When the pH of the electrolyte was raised to 12.0, a greater

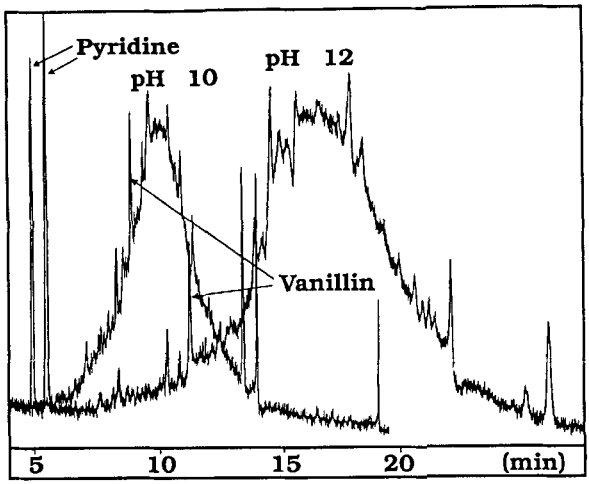

FIGURE 4. Electropherogram of underivatized dissolved lignin, GSK 4 (see table 1). The sample was separated at pH 10.0 and at pH 12.0. The increased mobility at pH 12.0 is due to an increased number of ionized phenolic groups. Conditions as in fig. 2.

number of phenolic groups were ionized, yielding a longer observed migration time (see equation [2]).

In CZE, the peak distribution becomes broader when compounds spend a prolonged time in the detector cell. This is most predominant for compounds with high observed migration time. The area should therefore always be corrected with respect to the observed migration time[32]. When characterizing lignins an increase in the pH also yields changes in the absorption spectra and extinction coefficients as a result of deprotonation of the lignin fragments and because of that the areas were not corrected in this study.

The increase in the observed migration time for the lignin at pH 12.0 is to some extent due to an observed decrease in EOF compared to that at pH

10.0. This is demonstrated by an increased observed migration time for the neutral marker in Figure 4. The decrease of EOF at pH 12.0 compared to pH 10.0 is in contradiction to the theory that predicts a slight increase. In our experiment, the observed decrease in EOF can be due to the zwitterionic glycine buffer. The obvious advantage of using this buffer in CZE is the very low resulting current (17 μA at 20 kV, pH 10 and 34 μA at 20 kV, pH 12), even though the concentration was 100 mM, which is considered high in CZE. Samples of liquor that contains lignin from the pulp industry, often also contain high concentrations of ions. Since peak shapes are affected by the ion concentration in the sample plug, it is important that the ionic strength of the electrolyte is higher than the ionic strength of the sample[33].

CONCLUSIONS

In this study, we have shown the applicability of CZE to the characterization of lignin. The electrophoretic mobility of dissolved kraft lignin reflects important parameters, charge and size, of the lignin dissolved during pulping. The mobility is increased during the cook and it is concluded that the phenolic groups play a more important role for the mobility than does the molecular size. According to this study, dissolved pulp lignin consists of fragments with different charge-to-size ratios. The charge distribution in the dissolved lignin becomes more even towards the end of the cook. This information provides a new dimension in the characterization of lignin.

With capillary zone electrophoresis it is possible to characterize kraft lignin using an alkaline electrolyte. The technique is simple to handle because little or no sample treatment is required. CZE is a new analytical tool which can be used as a complement to other techniques in an effort to learn more about the lignins in different pulp processes.

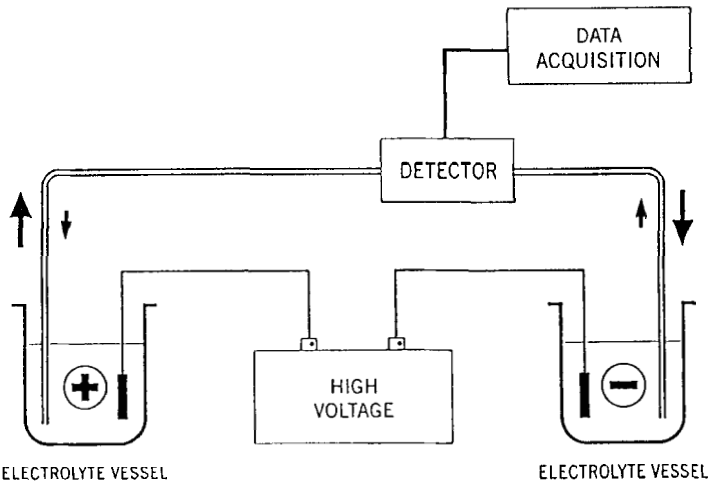

FIGURE 5. Schematic diagram of a capillary electrophoresis system showing the silica capillary, an on-column detector, the high voltage power connection and electrolyte reservoirs. The smaller arrows indicate the direction of the mobility of the anions and the larger arrows indicate the direction of the net flow caused by the EOF.

EXPERIMENTAL

Apparatus

All experiments were performed on an Applied Biosystem (Model 270A and Model 270A-HT) capillary electrophoresis system. A fused silica capillary, with a total length of 72 cm, 50 cm to the detector and an inner diameter of 50 μm was used. The basic principle of CZE is illustrated in Figure 5. The quartz capillary is filled with an appropriate electrolyte and immersed at both ends in electrolyte vessels. The injector consists of an automated vacuum sampling system. A small volume of the sample is introduced into the anodic end. A high voltage is applied which forces the components in the sample to migrate at different rates depending on their charge-to-weight ratio. Close to the other end of the capillary, a tunable on-column UV/ VIS-absorbance spectrophotometric detection is adopted.

The lignin samples were detected at 254, 280, 300, 320, 340, 360, 380, 400, 500 nm; in the case of sodium polystyrene sulphonates at 230 nm. After each run, the capillary was flushed with electrolyte while the UV-absorbance was monitored to ensure that there was no sample left in the capillary. A field strength of 278 V/cm was used which corresponds to a total effect of 0.34 W. This low effect excludes band broadening due to thermal gradients inside the capillary[26]. Data acquisition and calculations were carried out with a ELDS 900 (Chromatography Data Systems AB, Kungshög, Sweden).

Procedure

Freeze-dried lignin samples were dissolved in diluted electrolyte (1:1) and filtered through a 0.45 µm membrane filter (Chrompack, Acro disc LC 13) before use. This filtration procedure was further applied to all electrolytes. After preparation, samples were stored in a refrigerator under nitrogen. The electrophoresis was carried out at 20 kV with a temperature of 30 °C in the separation compartment. The capillary was flushed with 0.1 M NaOH and equilibrated for 4 min with the buffer used for separation before each run. To maintain the condition of the silica capillary, it was stored in distilled water. Because of this, aging of the capillary was considered to be of minor importance.

All chemicals were of analytical grade. 100 mM glycine buffers at pH 10.0 and 12.0, measured at room temperature, were used as electrolytes. Pyridine (0.01 % v/v) was used as a neutral marker. Sodium polystyrenes were purchased from Polymer Laboratories Ltd., UK.

ACKNOWLEDGMENT

The authors gratefully thank Prof. Göran Gellerstedt, Royal Institute of Technology, Department of Wood Chemistry, Stockholm, Sweden, who kindly provided the lignin samples used in this study.

REFERENCES

1. D.R. Robert, M. Bardet, G. Gellerstedt and E-L. Lindfors, J. Wood Chem. Technol., 4, 239 (1984).

2. M. Erickson, S. Larsson and G.E. Miksche, Acta Chem. Scand., 27, 903 (1973).

3. R. Mörck, K. Kringstad and P. Månsson, Canadian Wood Chemistry Symposium, Niagara Falls, Ontario September 13-15 (1982).

4. R. Mörck, H. Yoshida and K. Kringstad, Holzforschung, 40, 51 (1986).

5. S. Sarkanen, D.C. Teller, E. Abramowski and J.L. McCarthy, Macromolecules, 15, 1098 (1982).

6. J. Pellinen and M. Salkinoja-Salonen, J. Chromatogr,. 322, 129 (1985).

7. J. Pellinen and M. Salkinoja-Salonen, J. Chromatogr,. 328, 299 (1985).

8. S. Hjertén, Chromatogr. Rev., 9, 122 (1967).

9. J.W. Jorgenson and K.D. Lukacs, Anal. Chem., 53, 1298 (1981).

10. J.W. Jorgenson and K.D. Lukacs, J. Chromatogr., 218, 209 (1981).

11. T. Tsuda, K. Nomura and G. Nakagawa, J. Chromatogr., 264, 385 (1983).

12. X. Huang, J.A. Luckey, M.J. Gordon and R.N. Zare, Anal. Chem., 61, 766 (1989).

13. M.T. Ackermans, F.M. Everaerts and J.L.Beckers, J. Chromatogr., 549, 345 (1991).

14. B.F. Kenney, J. Chromatogr., 546, 423 (1991).

15. C.D. Gaitonde and P.V. Pathak, J. Chromatogr., 514, 389 (1990).

16. S. Hoffstetter-Kuhn, A. Paulus, E. Gassmann and H.M. Widmer, Anal. Chem., 63, 1541 (1991).

17. V. Pretorius, B.J. Hopkins and J.D. Schieke, J. Chromatogr., 99, 23 (1974).

18. K.D. Altria and C.F. Simpson, Chromatographia, 24, 527 (1987).

19. W.J. Lambert and D.L. Middleton, Anal. Chem., 62, 1585 (1990).

20. H.T. Rasmussen and H.M. McNair, J. Chromatogr., 516, 223 (1990).

21. H.J. Issaq, I.Z. Atamna, C.J. Metral and G.M. Muschik, J. Liq. Chromatogr., 13, 1247 (1990).

22. H.J. Issaq, I.Z. Atamna, G.M. Muschik and Janini, G.M., Chromatographia, 32, 155 (1991).

23. F.E.P. Mikkers, F.M Everaerts and Th.P.E.M. Verheggen, J. Chromatogr., 169, 1 (1979).

24. T. Satow, A. Machida, K. Funakushi and R. Palmieri, J. High Resolut. Chromatogr., 14, 276 (1991).

25. I.Z. Atamna, C.J. Metral, G.M. Muschik and H.J. Issaq, J. Liq. Chromatogr., 13, 2517 (1990).

26. Y. Kurosu, K. Hibi, T. Sasaki and M. Saito, J. High Resolut. Chromatogr., 14, 200 (1991).

27. S. Fujiwara and S. Honda, Anal. Chem., 59, 487 (1987).

28. C. Schwer and E. Kenndler, Anal. Chem., 63, 1801 (1991).

29. J.C. Giddings, Sep. Sci., 4, 181 (1969).

30. G. Gellerstedt and E-L. Lindfors, Holzforschung, 38, 151 (1984).

31. J. Gierer, personal communication.

32. X. Huang, W.F. Coleman and R.N. Zare, J. Chromatogr., 480, 95 (1989).

33. D.S. Burgi and R-L. Chien, Anal. Chem., 63, 2042 (1991).