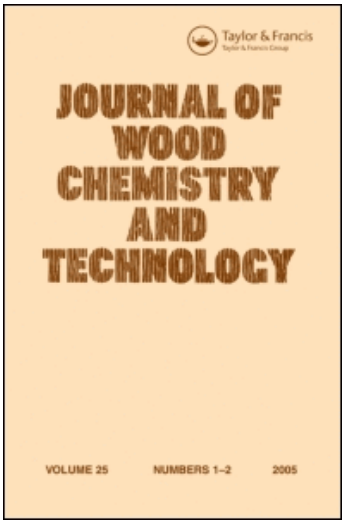

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AQUEOUS GEL PERMEATION CHROMATOGRAPHIC METHODS FOR TECHNICAL LIGNINS

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ABSTRACT

The molecular weights and molecular weight distributions of lignosulfonates and soda lignins were determined by aqueous gel permeation chromatography (GPC). The elution behavior of the samples, was investigated in detail to evaluate the non-size-exclusive effect in the aqueous GPC system. The ionic strength and pH are thought to be the main factors influencing the accuracy and the reliability of measurements. Solutions of 0.1M $NaNO_3$ with pH 7 or 0.1 M $NaNO_3$ with pH 12 were shown to be optimum for determining the molecular weight of lignosulfonate and alkali lignin. Accuracy of the method was checked by comparing the data obtained by aqueous GPC with that from vapor pressure osmometry (VPO).

INTRODUCTION

Technical lignins are major by-products produced in pulping in the papermaking industry. The water solubility and reactivity of lignin is increased significantly due to the introduction of hydrophilic groups and cleavage of linkages between structural units of lignin in the pulping process. This makes it further chemical modification possible and many kinds of useful chemicals can be manufactured from technical lignins.

As the molecular weight and molecular weight distribution are important factors determining of lignin, the measurement of molecular weights and molecular weight distributions has been reported since 1960s. Marton, *et al.*[1] succeeded in determining the molecular weight distribution of kraft lignin by VPO and equilibrium sedimentation in ultracentrifugation. Forss *et al* [2,3] successfully determined the molecular weight distribution of kraft lignin and lignosulfonate by gel permeation chromatography (GPC) in the 1960s. As both the molecular weight and the molecular weight distribution can be determined effectively, numerous investigators have used this method in the study of lignin chemistry. Kristersson[4] and Zakharov, *et al.*[5] studied GPC of lignin carbohydrate complexes (LCC) on Sephadex columns.

All these investigations were conducted on columns that were filled with "soft" gels. Due to their low performance, relatively long columns were needed. This resulted in such problems as long determination time and poor separation. To solve these problems, analysis using high performance GPC was developed. Van der Hage *et al.*[6] developed and optimized an aqueous high performance GPC method for lignosulfonates using a TSK G3000SW column.

The analysis of technical lignins by GPC can be conducted with either aqueous or non-aqueous mobile phases. Aqueous GPC for strongly hydrophilic lignins, e.g., lignosulfonates, has been reported in detail references. Because of the difficulty in obtaining standard samples with narrow molecular weight distributions, aqueous GPC has not been utilizes as extensively. For less hydrophilic technical lignins, the commonly used method is to elute the acetylated lignin sample with tetrahydrofuran (THF). Though accepted by most people, this method has some disadvantages such as the high cost of analysis and the inconvenience of pretreatment of samples. The samples should be acetylated before determination in order to increase the solubility in the eluent. In addition, eluents such as tetrahydrofuran (THF) are relatively expensive. Thus, aqueous GPC would be desirable if it could be widely used in the analysis of lignins. In this paper, determination of the molecular weights and the molecular weights distribution of lignosulfonates and alkali lignins by aqueous GPC are discussed.

RESULTS AND DISCUSSION

During aqueous GPC of polyelectrolytes, the retention volume of solutes depends not only on its hydrodynamic volume but also on other factors such ion exclusion, ion-inclusion, ion exchange and adsorption of solutes.[6] All of these non-size exclusion effects are sometimes named as secondary separation effects, and arise from the interaction between the molecules of polar and ionized solutes and mobile phase or between the solutes and the gels. Secondary separation effects may lead to a deviation in data obtained. Secondary separation effects in aqueous GPC have been observed and studied in literature.[7,8]

It is common knowledge that both kraft lignin and lignosulfonate contain some ionizable functional groups such as carboxyl, phenolic hydroxyl, and sulfonate groups. The elution behavior of lignin samples, not only the position of chromatographic peak but also the peak area and the resolution, can be affected by secondary separation effects. It is fortunate that these effects can be eliminated or reduced during the GPC measurements.

Ionic strength and pH are recognized as the main factors that influence the elution behavior of lignin samples. By adjusting the pH and the ionic strength, the secondary separation effects can be suppressed.[9] In our experiments, we optimized the measurement conditions by adjusting the ionic strength and pH value of the mobile phase.

Ionic strength

Chromatograms were recorded using solutions of different kinds of electrolytes or solutions of same kind of electrolyte but of different concentrations as mobile phases. The retention times, peak area and peak shapes were compared.

The data in Table 1 and Table 2 demonstrate that the retention time and the peak area are strongly dependent on the ionic strength. The data of ionic strength in Tables 1 and 2 were calculated using Lewis-Randall Equation. It was found that the retention time (or retention volume) tended to increase when an eluent of higher ionic strength was used. This indicates that the lignin molecule expands

TABLE 1
Influence of Ionic Strength on the Elution Behavior of Sodium Lignosulfonate

Mobile phase	pH value	Ionic strength	Retention time (min)	Peak area
0.1 M $NaNO_3$	6	0.1	13.9	399
0.2 M $NaNO_3$	6	0.2	15.4	444
0.05 M NaCl	6	0.05	13.9	313
0.1 M NaCl	6	0.1	14.9	328
0.2 M NaCl	6	0.2	15.8	386

TABLE 2
Influence of Ionic Strength on the Elution Behavior of Birch Soda Lignin

Mobile phase	pH value	Ionic strength	Retention time (min)	Peak area
0.1M $NaNO_3$	7.5	0.1	13.7	447
0.2M $NaNO_3$	7.5	0.2	14.4	463
0.4M $NaNO_3$	7.5	0.4	14.4	467
0.01M $NaNO_3$:CH_3CN (8:2)	7.5	0.008	12.8	387
0.1M $NaNO_3$:CH_3CN (8:2)	7.6	0.08	13.3	402

due to the static electric repulsion between functional groups at low ionic strength. As the hydrodynamic volume is increased, penetration or diffusion of the molecules of the solutes into the porous gel is restricted. Earlier chromatogram could thus be observed.

It is observed from Table 1 that chromatograms with larger peak areas were obtained if an eluent with higher ionic strength was used. This indicates that the adsorption of solute could be reduced at higher ionic strength.

The data in Table 1, together with those in Table 2, also indicate that a pair of opposite factors, i.e., adsorption and ionic repulsion, simultaneously act on the position of the chromatographic peak of lignin.

It is also observed that the difference in the electrolytes, that is, $NaNO_3$ and NaCl, within the solution results in different retention-time and peak area data. A solution of $NaNO_3$ can lead to relatively shorter retention times and relatively larger peak areas than that of NaCl of the same concentration and pH. It is evident

TABLE 3
Influence of pH Value on Elution Behavior of Soda Lignin

Mobile phase	pH value	Ionic strength	Retention time (min)	Peak area
0.01M NaOH	12	0.01	13.40	585
0.001M NaOH	10	0.001	13.02	548
0.01M $NaNO_3$:CH_3CN	7.5	0.008	12.80	387

that the two electrolytes have different influences on the elution behaviors of lignin though they are both mono-valent inorganic salts.

In general, improved peak shape and fractionation can be observed when a mobile phase of relatively high ionic strength is used. This can be explained in terms of suppression of ionic exclusion at higher ionic strengths. At a higher concentration of electrolytes, interaction between lignin molecules is suppressed. The hydrodynamic volume of lignosulfonate or kraft lignin tended to decrease. The retention time (or retention volume) of the samples thus tended to increase. The phenomenon is in good agreement with the literature.[6]

pH value

It was reported that fractionation of technical lignin, as with many macromolecular compounds, is highly dependent on the pH of the eluent.[3]

In this study a series of eluents having different pH values was used on the same column to evaluate the influence of pH on the elution behavior of standard samples and lignin samples. The effects of pH value on the elution behavior of soda lignin and lignosulfonate are shown in Tables 3 and 4. The calibration lines of PEG eluted by the mobile phases of the same composition but different pH values were show in Figure 1.

Barth[10] has reported the influence of pH on the elution volume of lignosulfonate. Chromatograms in our experiments (Figures 2 & 3) indicate that a $NaNO_3$ solution of relatively high pH value (e.g., pH=8) can provide a better separation of lignosulfonate samples when it is used as the eluent. The peak area of the chromatogram at higher pH is bigger than that at a lower pH, indicating that

TABLE 4
Influence of pH Value on Elution Behavior of Sodium Lignosulfonate

Mobile phase	pH value	Ionic strength	Retention time (min)	Peak area
0.1M $NaNO_3$	6	0.1	13.9	399
0.1M $NaNO_3$	7	0.1	13.6	442
0.1M $NaNO_3$	8	0.1	13.5	744

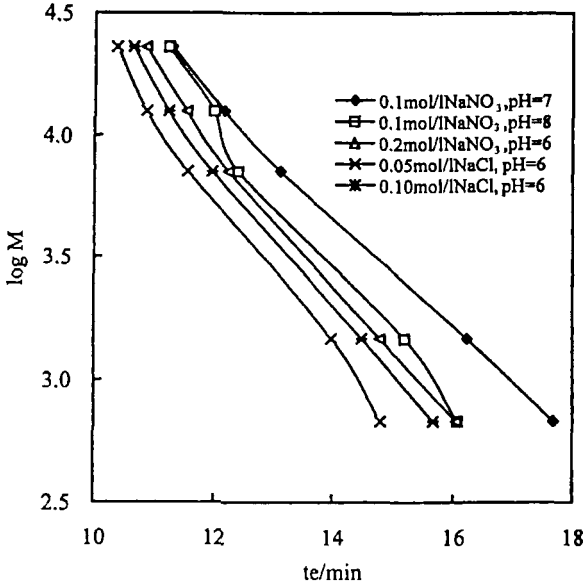

FIGURE 1. Influences of pH value on retention time (t_e) of PEG.

adsorption of the solutes is suppressed at relatively high pH. However, the elution profile of standard samples at higher pH becomes wider than that at lower pH and the linear relationship of log M versus t_e line is not maintained. On the other hand, improved chromatographic resolution of standard samples was observed when a $NaNO_3$ solution of lower pH was used as the eluent; the peak area of the lignin samples tended to reduce. In considering the demands for good separation of

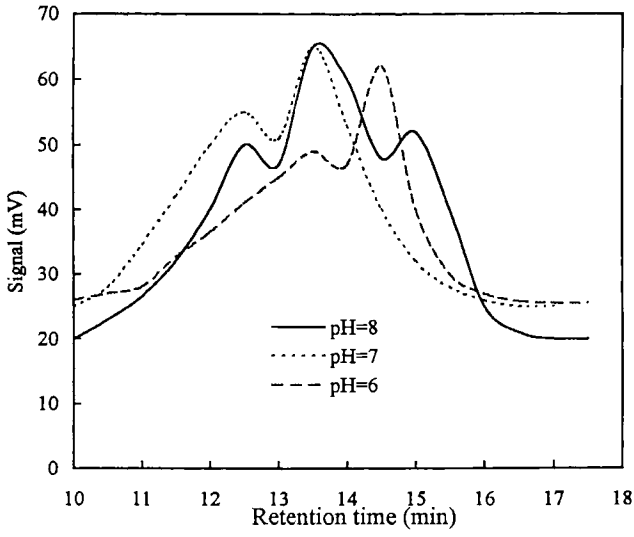

FIGURE 2. Chromatogram of sodium lignosulfonate at different pH values (eluted by 0.1M $NaNO_3$).

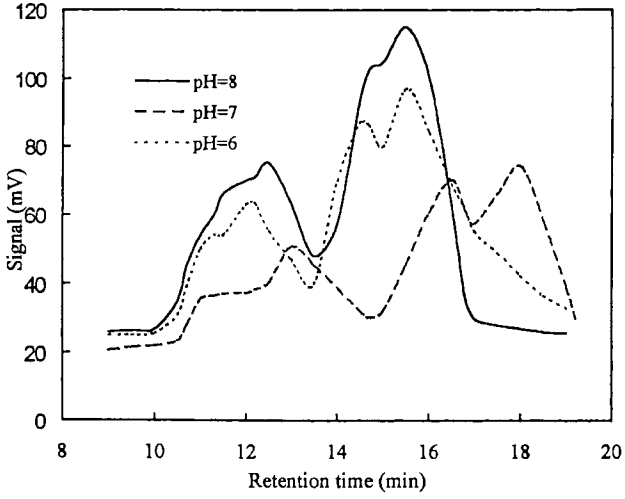

FIGURE 3. Chromatogram of PEG at different pH values (eluted by 0.1M $NaNO_3$).

lignin samples and standard samples, a solution of 0.1M $NaNO_3$ of pH 7 was selected as the mobile phase in determining the molecular weight distribution of lignosulfonates.

Similarly, for alkali lignin and kraft lignin, the elution curve shifts towards that of non-electolytes of the same molecular weight when the pH is decreased.[6] The increase in elution volume at low pH is probably due to suppression of ion exclusion.

The optimum mobile phase pH using 0.01M NaOH solution was 12.

Though a higher pH might lead to less adsorption of solute on the column, the life of Ultrahydrogel, a cross-linked hydroxylated polymer with residual carboxyl groups, might be reduced.

The water solubility of soda lignin and kraft lignin is decreased at lower pH values. The fractions of alkali lignin with high molecular weight can not be dissolved completely in water when the pH value is below 10. Though it was found that alkali lignin is water soluble at relatively low pH after dissolving it in dilute alkali solution and then adjusting the pH value with neutral solvent, we prefer to determine the molecular weight distribution in alkaline medium.

If an eluent of low pH value was used, no significant increase in column pressure was observed. The peak area, however, was reduced, indicating that part of the solute was adsorbed on the column (see Table 3).

The adsorption is probably results from the interaction of hydrogen bonding between Ultrahydrogel and lignin. The hydrogen bonding would be strengthened at low pH and lead to severe adsorption of lignin in the column.

Too high a pH, however, will shorten the life of the gel. It is recommended that the pH of the mobile phase be in the range 10~12.

Calibration line

The calibration line was obtained from Pullulan. The molecular weights of the standard samples were 23000, 12600, 7100, 1470 and 600. It can be seen from Figure 4 that there was a good linear relationship between the logarithm of the molecular weight and the retention time. The straight line in Figure 4 was

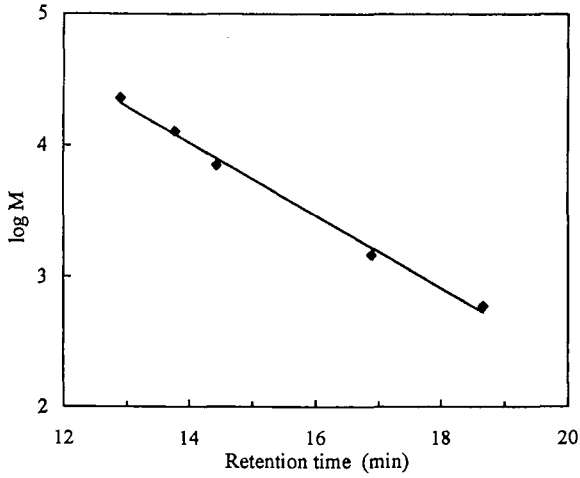

FIGURE 4. Calibration line of Pullulan.

alsoextrapolated so that the samples of smaller molecular weight could be determined. However, some accuracy might have been sacrificed by doing this. As Pullulan is a non-electrolyte, the calibration line is independent of the ionic strength, *i.e.*, this linear uncharged polymer is not subject to ionic strength.[7]

Reliability of aqueous GPC

The of molecular weight data for several lignin samples determined by two different methods are listed in Table 5. The data in Table 5 indicate that the number average molecular weights determined by the two methods represent good agreement. As sulfurized birch kraft lignin can neither not be dissolved, nor its acetate can be used, its molecular weight distribution can not be determined by non-aqueous gel permeation chromatography. The number average molecular weight was determined by VPO for comparison with aqueous GPC. Similar values of number average molecular weight were obtained by VPO. Thus, aqueous GPC can provide reliable molecular weights and molecular weight

TABLE 5
Molecular Weight of Soda Lignins and Kraft Lignins

Sample	Data obtained by Non-aqueous GPC			Data obtained by aqueous GPC		
	$\overline{M}_n$	$\overline{M}_w$	d	$\overline{M}_n$	$\overline{M}_w$	d
Eucalyptus soda lignin	5900	12600	2.14	5470	11660	2.13
Eucalyptus soda lignin	7700	14600	1.90	7600	13740	1.86
Birch kraft lignin	——	——	——	7523	19650	2.69
Sulfurized birch kraft lignin	——	——	——	5839	10790	1.92
Sulfurized birch kraft lignin	——	——	——	5455	9914	1.82

TABLE 6
Molecular Weights of Lignosulfonates Determined by Aqueous GPC

Sample	$\overline{M}_n$	$\overline{M}_w$	d	$\overline{M}_n$ by VPO
Sodium lignosulfonate	3441	7082	2.05	3405
Calcium lignosulfonate	8691	29038	3.36	8564
Sulfonated soda lignin	5037	10438	1.86	5095

distributions of technical lignin samples. In addition, it is a simple and reliable method.

EXPERIMENTAL

Materials

Sodium lignosulfonate and calcium lignosulfnate from *pinus massion* are commercial products of Guangzhou Paper Mill. Birch kraft lignin, eucalyptus soda lignin and their surfuration products were provided by Department of Chemical Engineering, Tianjin Institute of Light Industry. Wheat straw soda lignin and sulfurized wheat straw soda lignin were obtained from State Key Laboratory of Pulp and Paper Engineering, South China University of Technology.

Pullulan, a narrow-distribution polysaccharide, and PEG, a narrow-distribution polyethylene glycol, both produced by Shedex Standard Company, USA, were used as the standard samples.

NaCl, NaOH, $NaNO_3$, tetrahydrofuran and acetylnitrile were of A.R. grade. Acetylnitrile and tetrahydrofuran were distilled to remove impurities. Water was treated by a Milli-Q 50 ultra-pure water system manufactured by Millipore Company.

Instrumentation

The instrumentation was a Waters 244 GPC/HPLC chromatogram, fitted with a 6000A solvent-delivery system, a U6k injection valve, a Waters Ultrahydrogel 250 column covering the molecular weight separation range 1000~80000, an Ultrastyragel 1000 column covering the molecular weight separation range 2000~30000 and a Waters 410 differential refractometer.

Procedures

The lignin samples, as well as standard samples, were dissolved in the mobile phase and then filtered through Millipore HA membrane (0.45 μm) to remove any suspended materials. Approximately 100 μL of the solution having a concentration of approximate 3 mg/mL was injected into the column in sequence. The flow rate was controlled at 0.6 mL/min. The elution profile was recorded with Baseline 810 interface and data processing software. The molecular weight and the molecular weight distribution were calculated using to the logM versus t_e line of the standard samples.

CONCLUSIONS

The molecular weights and molecular weight distributions of soda lignin, kraft lignin, together with their sulfurized products, and lignosulfonates, can be determined by aqueous GPC.

The secondary separation effects in aqueous GPC can be minimized under suitable conditions.

The data from aqueous GPC are in good agreement with those obtained by non-aqueous GPC methods. That means that aqueous GPC is reliable in determining molecular weight and molecular weight distribution of kraft lignin and soda lignin. Aqueous GPC shows its unique advantage for those lignin samples that can not be acetylated.

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