Chapter 9

Determination of the Molar Mass Distribution of Lignins by Gel Permeation Chromatography

Kaj Forss, Raimo Kokkonen, and Pehr-Erik Sågfors

The Finnish Pulp and Paper Research Institute, P.O. Box 136, 00101 Helsinki, Finland

This paper describes the fractionation of lignin sulfonates on elution through Sephadex G-50, G-75 and Sephacryl S-300 using water or a 0.5M sodium chloride solution buffered to pH 8 as the eluent. Fractionation of kraft lignin on elution through Sephadex G-50 with 0.5M sodium hydroxide as well as the influence of the sodium hydroxide concentration is also described. By comparing the retention volumes of proteins and lignin sulfonate fractions with known molar masses, it is shown that several commercially available proteins can be used for calibration of the columns. It is shown that on elution through Sephadex G-25 with 0.5M sodium hydroxide the retention volume of monomeric compounds is influenced more by their functional groups than by their molecular size.

A method is needed for the determination of the molar mass and molar mass distributions of lignins. The method should give reproducible results when used in different laboratories. The procedure should not be complicated, and the calibration components must be readily available and reasonably priced.

The present paper describes the fractionation of lignin sulfonates and kraft lignin by gel permeation chromatography (GPC) and the method developed and used for several years at the Finnish Pulp and Paper Research Institute.

Sulfonated Lignins

Elution of Lignin Sulfonates with Water. As can be seen from Figures 1, 2, 3, and 4, the fractionation of lignin sulfonates on elution with water through Sephadex G-50 and G-75 takes place in such a way that the logarithms of

0097–6156/89/0397–0124$06.00/0

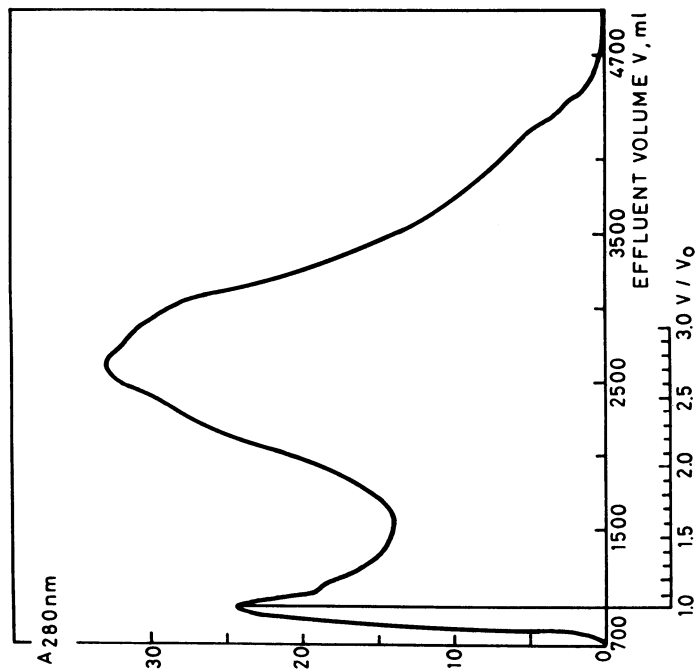

Figure 1. Fractionation of lignin sulfonates in spent sulfite liquor on Sephadex G-50. Eluent: water. (Reprinted with permission from ref. 1. Copyright 1969 Wiley.)

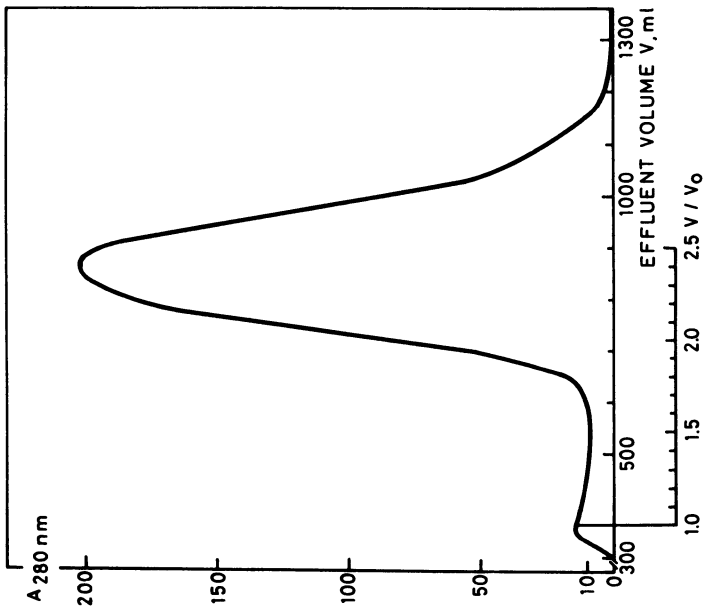

Figure 2. Fractionation of lignin sulfonates in spent sulfite liquor on Sephadex G-75. Eluent: water. (Reprinted with permission from ref. 1. Copyright 1969 Wiley.)

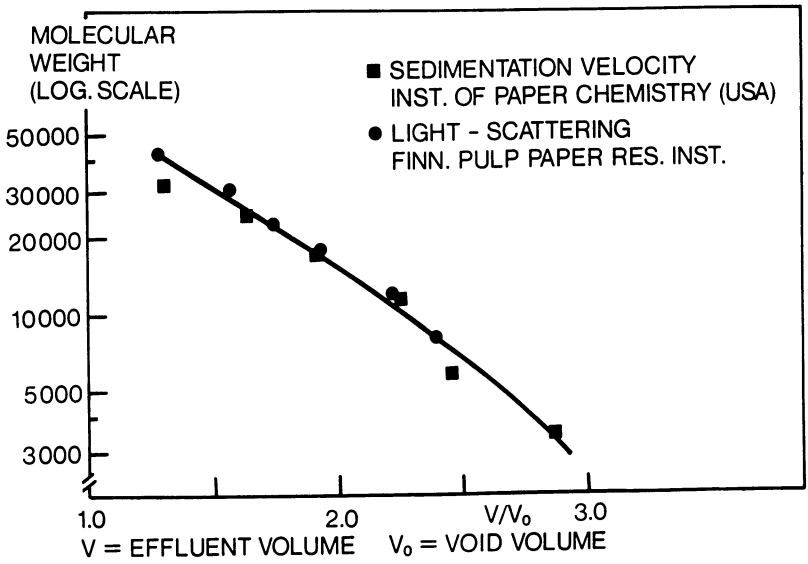

Figure 3. Molar mass as a function of retention volume for lignin sulfonates. Column: Sephadex G-50. Eluent: water. (Reprinted with permission from ref. 1. Copyright 1969 Wiley.)

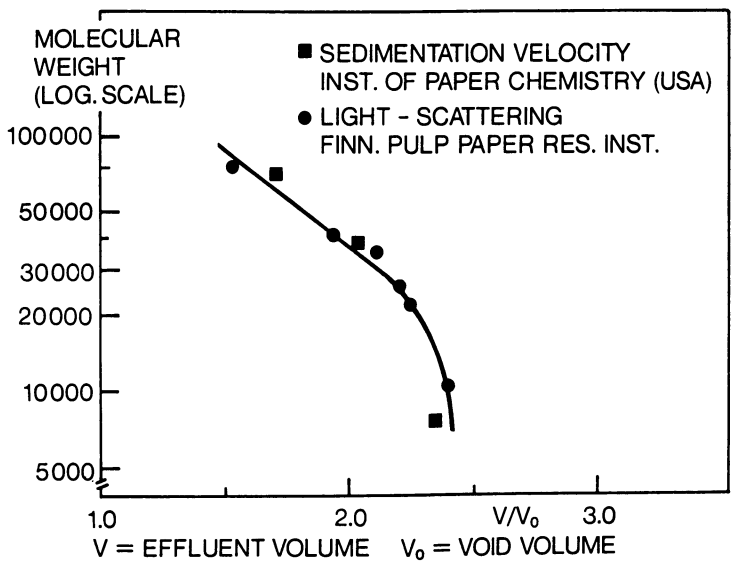

Figure 4. Molar mass as a function of retention volume for lignin sulfonates. Column: Sephadex G-75. Eluent: water. (Reprinted with permission from ref. 1. Copyright 1969 Wiley.)

the molar masses of the lignin sulfonates show a straightline relationship with the retention volumes (1).

In these experiments Sephadex G-50 effected fractionation of the lignin sulfonates with molar masses of 5 000–50 000 dalton and Sephadex G-75 those with molar masses of 20 000–100 000 dalton. The molar mass determinations carried out using the light-scattering and ultracentrifugal sedimentation velocity methods gave identical results. However, calibrating the columns using light-scattering or ultracentrifugation is too awkward for routine determinations. There is thus a need for readily available calibration standards of known molar mass that elute at the same retention volume as lignin sulfonates of the same molar mass.

It should be noted that the relationships between molar mass and retention volume for lignin sulfonates shown in Figures 3 and 4 are strictly only valid for the samples studied in these experiments because lignin sulfonates are polyelectrolytes and thus interact with each other and with the gel matrix of the column. The shape of the calibration curve is thus affected by, among other things, the size and concentration of the sample (2). Interactions between molecular species can be eliminated by eluting with a suitable electrolyte.

Elution of Lignin Sulfonates with Electrolyte Solution and Calibration of Columns

The effects caused by the electrolyte nature of lignin sulfonates are eliminated by using a 0.5M sodium chloride solution as eluent. This eluent is made 0.1M with respect to Tris-HCl and buffered to pH 8 with hydrochloric acid in order to dissolve the proteins used as calibration standards (Fig. 5).

The column is calibrated using proteins of known molar mass. The relative retention volumes 0.0 and 1.0 are defined by the elution of Blue Dextran (molecular weight 2 000 000) and sulfosalicylic acid (molecular weight 218), respectively.

In the experiment described in Figure 6, four lignin sulfonate fractions with known molar masses were eluted.

Figure 6 shows that the proteins used for calibration elute in the same way as lignin sulfonates, which justifies the use of proteins as calibration standards. A comparison between Figures 4 and 6 shows that elution with an electrolyte solution fractionates lignin sulfonates in the range 3 000–80 000 dalton, but that elution with water fractionates those in the range 20 000–100 000 dalton.

Because of the very high molar masses of enzymically polymerized lignin sulfonates, Sephacryl S-300 is used as the gel matrix. The fractionation range is 10 000 to 1 000 000 dalton. Even in this case proteins of known molar mass can be used as calibration standards (Figs. 7 and 8).

One disadvantage of using salt solution as eluent is that the lignin sulfonates tend to adsorb onto the gel matrix, resulting in a resolution inferior to that obtained by elution with water. On the other hand, elution behavior with water is adversely affected by the polyelectrolyte properties of the lignin sulfonates. Adsorption, which is caused by the phenolic hydroxyl

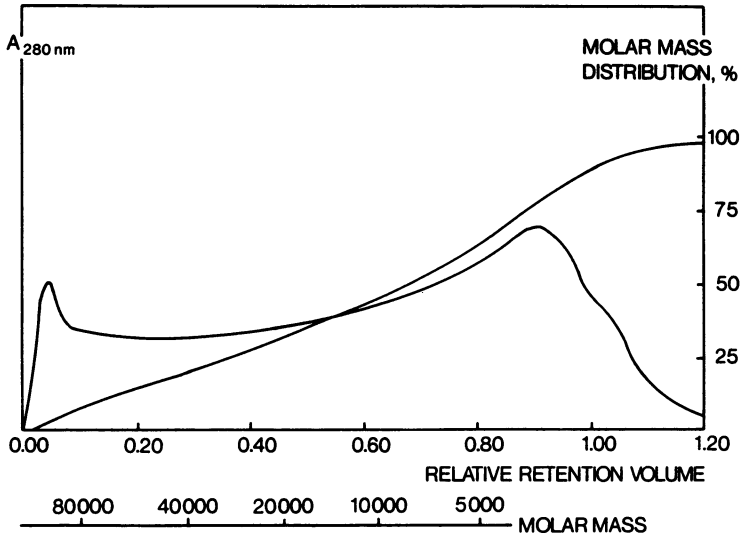

Figure 5. Fractionation of lignin sulfonates on elution through Sephadex G-75. Eluent: 0.5M NaCl, 0.1M Tris-HCl (pH 8).

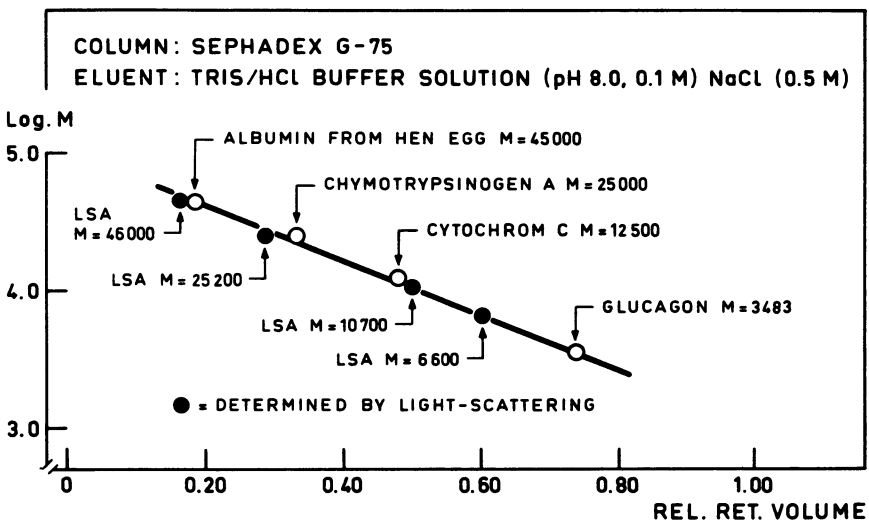

Figure 6. Calibration of Sephadex G-75. Eluent: 0.5M NaCl, 0.1M Tris-HCl, pH 8 (4).

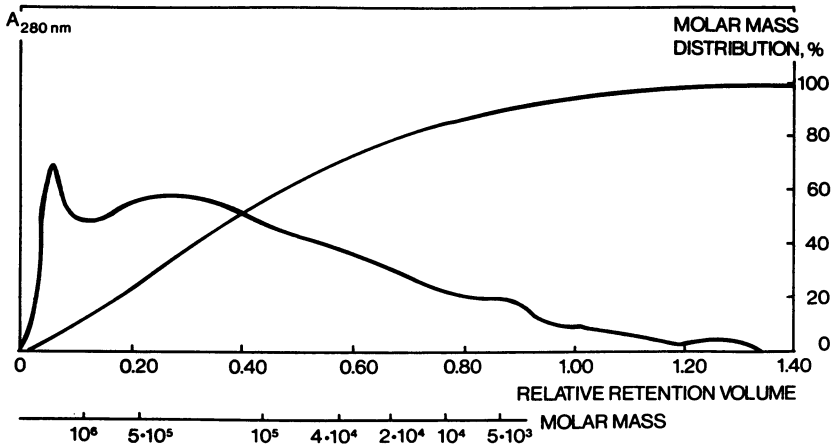

Figure 7. Fractionation of enzymically polymerized lignin sulfonates through Sephacryl S-300. Eluent: 0.5M NaCl, 0.1M Tris-HCl (pH 8).

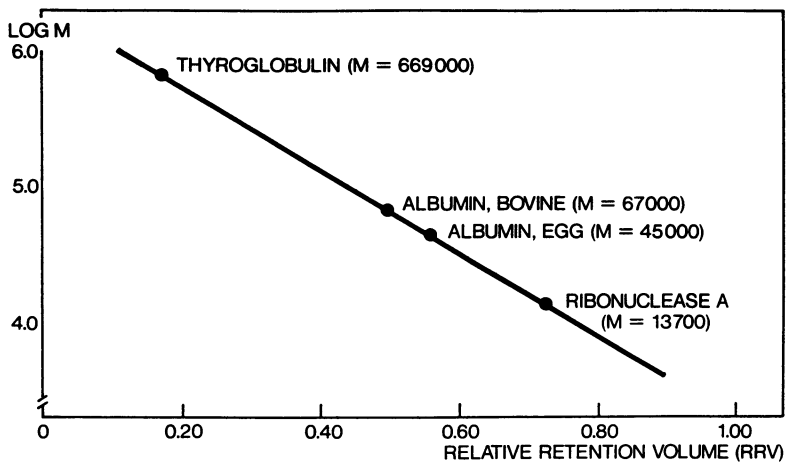

Figure 8. Calibration of Sephacryl S-300. Eluent: 0.5M NaCl, 0.1M Tris-HCl (pH 8).

groups of the lignin sulfonates, can be avoided by using a strongly alkaline eluent to ionize the phenolic hydroxyl groups. However, the Sephadex G-75 used for the fractionation of lignin sulfonates in the molar mass range 3 000–80 000 dalton is rapidly degraded by strong alkaline solutions. Sephacryl is stable enough, but the high molar mass calibration proteins cannot be used in strong alkaline solution.

Elution of Non-Sulfonated Lignins with Sodium Hydroxide Solution as Eluent

Non-sulfonated lignins such as those from alkaline pulping processes are insoluble in water but easily soluble in sodium hydroxide solutions. When dissolved in and eluted with a sodium hydroxide solution, they show polyelectrolyte properties, i.e., the molecular species interact. As revealed by Figure 9, the fractionation result is strongly dependent on the sodium hydroxide concentration up to a concentration of 0.4M. A 0.5M sodium hydroxide solution is thus an appropriate eluent for fractionation on Sephadex G-50 (3).

With 0.5M sodium hydroxide as eluent, Sephadex G-50 effects fractionation in the molar mass range 1000–15000 dalton and can be used for a period of 3-4 weeks with a single calibration carried out with proteins and polypeptides of known molar mass, as revealed by Figure 10. Relative retention volumes 0.0 and 1.0 are defined with Blue Dextran and phenol, respectively.

Due to the dark color of alkali lignins, their molar masses cannot be determined by means of the light-scattering method. However, as shown by Figure 10, elution with sodium hydroxide also brings about a consistent elution pattern of lignin sulfonates and polypeptides. It is assumed that this also applies to the kraft lignins.

Fractionation on Sephadex G-25 using 0.5M sodium hydroxide as eluent causes the low molar mass lignin components in black liquor to elute in the relative retention volume range 0.3-1.3 with partial separation from each other, as shown in Figure 11.

It should be noted that in this relative retention volume range elution does not necessarily take place in order of decreasing molecular size, because, as seen from Figure 12, functional groups may have a greater effect on elution behavior than molecular size (Forss, K.; Talka, E., The Finnish Pulp and Paper Research Institute, unpublished results).

Summary

It has been shown that the molar mass distributions of lignin sulfonates and kraft lignin can be determined by gel permeation chromatography. Calibration of the columns with lignin sulfonates of known molar mass or, alternatively, with commercially available proteins and polypeptides has been shown to give the same result.

Because of the polyelectrolyte properties of lignins, elution is performed with electrolyte solutions. If the lignins are water soluble and the column

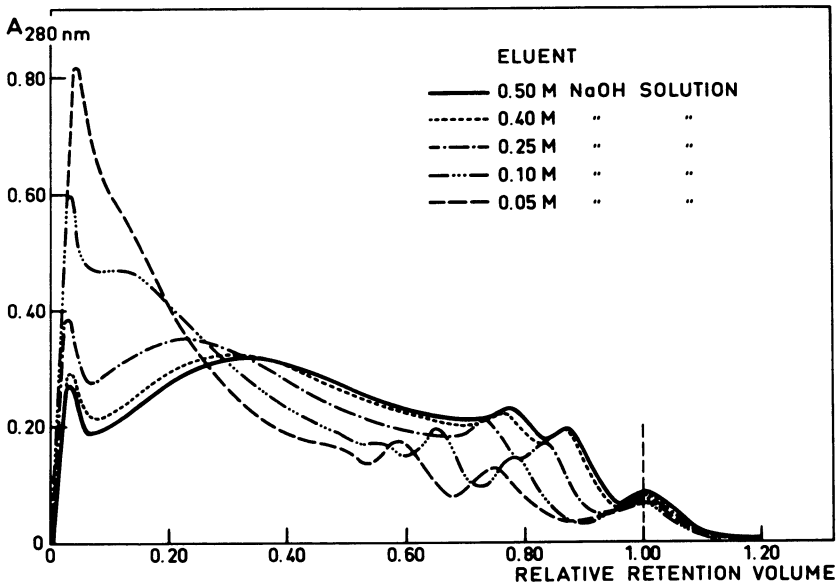

Figure 9. Influence of sodium hydroxide concentrations in the eluent on fractionation of lignins in draft black liquor. Column: Sephadex G-50. (Reprinted with permission from ref. 3. Copyright 1976 Wiley.)

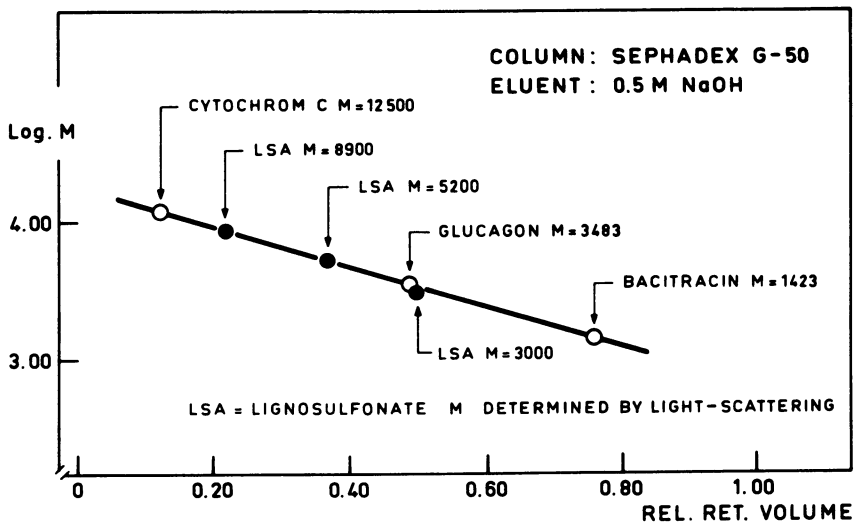

Figure 10. Calibration of Sephadex G-50. Eluent: 0.5M NaOH (4).

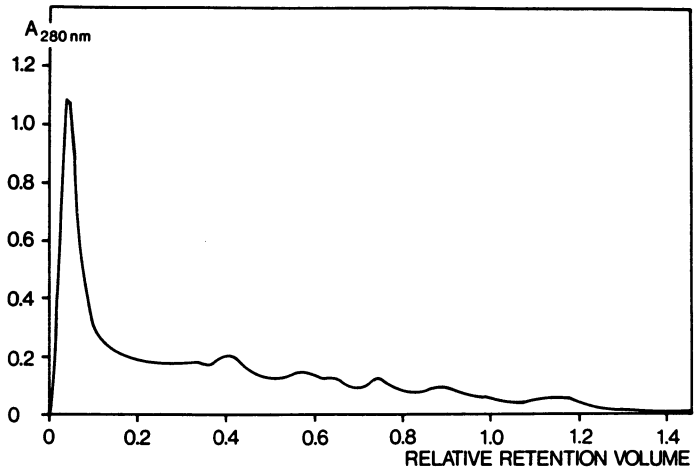

Figure 11. Fractionation of low molar mass lignin components in kraft black liquor. Column: Sephadex G-25. Eluent: 0.5M NaOH.

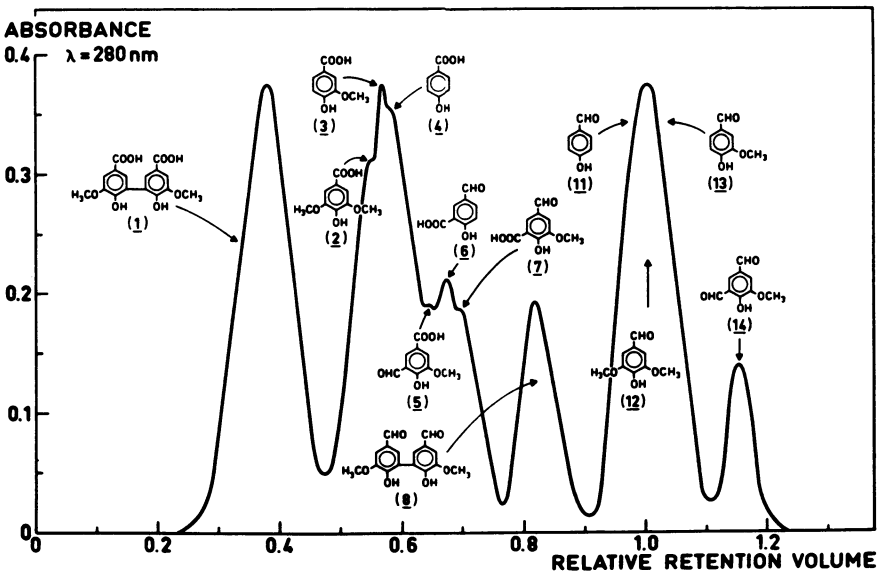

Figure 12. Fractionation of low molar mass model compounds. Column: Sephadex G-25. Eluent: 0.5M NaOH.

gel matrix is not stable enough in the presence of an alkaline eluent, the elution is performed with 0.5M sodium chloride solution adjusted to pH 8 with 0.1M Tris-HCl buffer. If the lignins are soluble only in alkaline solution the elution is performed with 0.5M sodium hydroxide solution.

Literature Cited

1. Forss, K.; Stenlund, B. *Pap. Puu* 1969, **51**, 93-105.
2. Stenlund, B. *Pap. Puu* 1970, **52**, 55-62; 121-30; 197-206; 333-39; 671-79.
3. Forss, K.; Stenlund, B.; Sågfors, P.-E. *Appl. Polym. Symp.* 1976, **28**, 1185-94.
4. Forss, K. G.; Furmann, A. G. M. U.S. Patent 4 105 606, 1978.

RECEIVED April 3, 1989