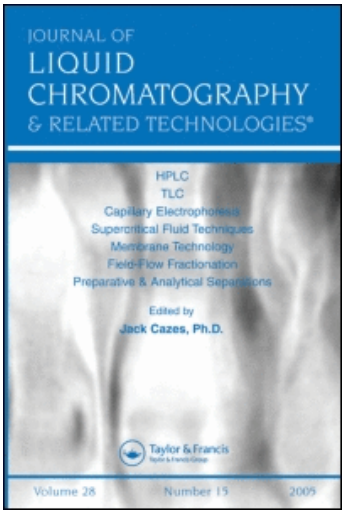

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Elution Behavior of Oligomers on a Polyvinyl Alcohol Gel Column with Chloroform, Methanol, and Their Mixtures

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ELUTION BEHAVIOR OF OLIGOMERS ON A POLYVINYL ALCOHOL GEL COLUMN WITH CHLOROFORM, METHANOL, AND THEIR MIXTURES

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ABSTRACT

Elution phenomena of size exclusion chromatography (SEC) plus superimposed adsorption effects for oligostyrenes, epoxy resins, methylated melamine-formaldehyde resin prepolymers, p-cresol-formaldehyde resin prepolymers, and phenol-formaldehyde resin prepolymers were investigated. SEC and superimposed adsorption effects could be elucidated from a concept of solubility parameter. Minimum retention volumes of these oligomers were obtained with the mobile phases of chloroform/methanol, 80/20 or 60/40 (v/v), and separation was expected to be mostly performed by SEC. The solubility parameter of polyvinyl alcohol gels was estimated to be between 21 and 23 from the above results. Elution for normal phase chromatography was in the order of increasing molecular weight and that for reversed-phase chromatography was in the order of decreasing molecular weight. These are reversed phenomena to those for low-molecular weight compounds. Solubility of sample solutes to mobile phase must be considered. Methanol mobile phase-polyvinyl alcohol gel system might be exception.

INTRODUCTION

Polyvinyl alcohol gels, which are now commercially available, are hydrophilic in nature, and therefore, they are exclusively used for aqueous size exclusion chromatography. Mobile phases used in a column packed with these gels are aqueous solutions or those plus small amount of organic solvents. Recently, a report showed that some types of polyvinyl alcohol gels were compatible with polar and non-polar organic solvents[1]. It means that columns packed with these gels can be used with both polar and non-polar organic solvents as mobile phases without loss of the column efficiency.

In this paper, chloroform, methanol and their mixtures were used as mobile phases and elution behavior of several oligomers on a polyvinyl alcohol gel column was investigated, focusing size exclusion chromatography (SEC) plus superimposed secondary effects.

EXPERIMENTAL

An Asahipak GS-310 column (7.6 mm I.D. x 500 mm) (Asahi Chemical Co., Kawasaki, 210, Japan) packed with polyvinyl alcohol (PVA) gels was used in this experiment. The exclusion limit of this column was about 20000 as polystyrene molecular weight and the maximum number of theoretical plates was 24000 plates by injecting 0.025 ml of 0.5% ethylene glycol solution in water and by using water as mobile phase. A liquid chromatograph was a Jasco (Japan Spectroscopic Co., Hachioji, Tokyo 192, Japan) TRIROTAR-V high performance liquid chromatograph with a Jasco ultra-violet (UV) detector Model UVIDEC-100 IV operated at 254 nm. Sample solutions were injected using a variable loop injector Model VL-611 (Jasco).

Mobile phases were chloroform, methanol and their mixtures, and elution was performed by the isocratic mode. A flow rate was

0.5 ml/min. Chloroform used here included 1% ethanol as a stabilizer in advance. Concentration of sample solutions was 0.5% and the injection volume of the sample solutions was 0.1 ml. Samples were dissolved in the solvent used as the mobile phase.

Sample oligomers were oligostyrene A-500 (avearge molecular weight was 500), epoxy resins, EPIKOTE 828 and 1001, methylated melamine-formaldehyde resin prepolymers, p-cresol-formaldehyde resin prepolymers (novolac and resol types), and phenol-formaldehyde resin prepolymers (novolac type). Benzene was also used as a sample solute. Epoxy resins and p-cresol-formaldehyde resin prepolymers were not dissolved in mobile phases of chloroform/methanol, 20/80 and 0/100 (v/v), so that there were no results for these cases.

RESULTS and DISCUSSION

Table I shows the shifts of retention volume, the increase of peak width at half height, and the variation of the number of theoretical plates of a benzene solute with the increase of methanol content in chloroform used as mobile phases. Minimum reten-

TABLE 1

Retention Volume (V_R), Peak Width at Half Height ($W_{1/2}$), and Number of Theoretical Plates (N) of Benzene at Different Composition of Mobile Phases

	Mobile Phase					
	Chloroform/Methanol, (v/v)					
	100/0	80/20	60/40	40/60	20/80	0/100
V_R (ml)	13.55	13.58	14.90	16.40	17.92	19.72
$W_{1/2}$ (ml)	0.232	0.253	0.256	0.295	0.319	0.360
N	18900	16000	18700	17100	17500	16600

tion volume and peak width were obtained when chloroform was used as mobile phase. The increase of retention volume and peak width with the addition of methanol to chloroform used as mobile phase may be attributed to the adsorption effects superimposed on the size exclusion effect.

Chromatograms of oligostyrene A-500 are shown in Figure 1. Minimum retention volume was obtained at the composition of the mobile phase, chloroform/methanol, 80/20 (v/v). Resolution between each oligomer for chromatograms obtained with mobile phases chloroform/methanol, 80/20, 60/40, and 40/60, was similar and better than that with chloroform only. At mobile phase of chloroform/methanol, 20/80 (v/v), retention volume increased and resolution into peaks was not observed. Similarly, chromatogram obtained with the mobile phase of methanol was broad, no resolution was observed and peak retention volume increased to 18.2 ml. In the oligostyrene/chloroform-methanol system, separation is mainly by size exclusion and adsorption effects are superimposed on size exclusion effect. It might be hard to conclude that pure size exclusion would be expected when mobile phase of chloroform/methanol, 80/20 (v/v) is used, but it can be said that adsorption effects are minimized at this mobile phase.

According to a theory to elucidate the elution mechanism of size exclusion plus secondary effects, a concept of a solubility parameter can be applied[2]. Next equation can be used to estimate qualitatively whether the separation is performed by only size exclusion effect, or adsorption or partion effects are superimposed on size exclusion effect.

$$\log K \propto [(\delta_{solvent} - \delta_{gel})(\delta_{solvent} - \delta_{solute})] \quad (1)$$

where K is the partition coefficient for adsorption or partition effects, δ is solubility parameter designated by suffix for a solvent, a gel, and a solute, respectively. When the solubility parameter of solvent is equal to that of gel or a solute, separation of the solute is mainly performed by size exclusion effect

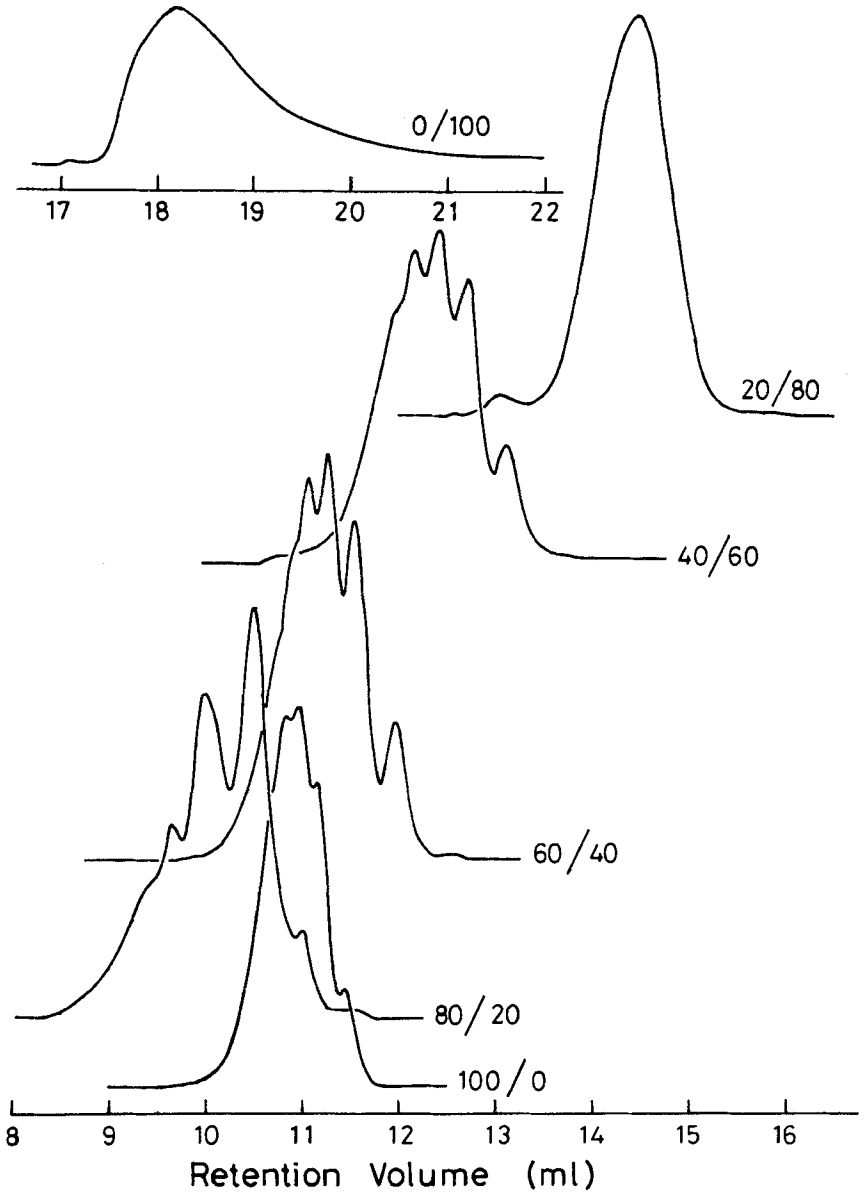

FIGURE 1. Chromatograms of oligostyrene A-500 (average molecular weight 500) at different composition of mobile phases. Composition of mobile phase: figures near chromatograms represent composition of chloroform/methanol as vol/vol.

and K is unity. This equation is useful for qualitative estimation of the degree of size exclusion effect and can be applied to non-ionic substances for solutes, gels, and solvents.

In other cases, secondary effects such as adsorption or partition effects must be superimposed on size exclusion, or an extremely case is that adsorption or partition effects are predominant for separation of solutes. In the case of a benzene solute, the solubility parameter of benzene is 18.6 $[J/cm^3]^{1/2}$ and that of chloroform is 18.8. Therefore, when chloroform was used as mobile phase, size exclusion was the main separation mechanism. The increase of methanol content to chloroform resulted in the increase of the adsorption or partition effects, and retention volume and peak width increased because of these secondary effects.

In cases of samples other than benzene, the minimum retention volumes were obtained when chloroform/methanol, 80/20 (v/v) (solubility parameter 20.9) or 60/40 (v/v) (solubility parameter 22.9) was used as the mobile phase. These results suggest that the solubility parameter of PVA gels may be between 21 and 23 rather than 25.8 appeared in the literature[3]. Therefore, it might be adequate not to be observed the increase of retention volume of benzene with the mobile phase of chloroform/methanol, 80/20 (v/v).

Minimum retention volumes of oligostyrene solutes were obtained at the mobile phase of chloroform/methanol, 80/20 (v/v) and resolution of the chromatogram was also better than that at the mobile phase of chloroform. Solubility parameter can be divided into three sub-solubility parameters; dispersion, polar, and hydrogen bonding. PVA gels have a strong hydrogen bonding character and the addition of small amount of methanol, which is also a strong hydrogen bonding solvent, might be effective to prevent adsorption effects on the surface of PVA gels with solutes. Excess addition of methanol to chloroform resulted in the increase of adsorption effects. Up to the mobile phase of chloroform/methanol, 40/60 (v/v), larger molecules eluted first. At the mobile phase of chloroform/methanol, 80/20 (v/v), elution

was retarded and peak became rather sharp. When methanol was used as the mobile phase, the chromatogram was distorted and became broard. Elution was also more retarded. The exchange of elution order between larger and smaller molecules occurred at the mobile phase of chloroform/methanol, 20/80 (v/v) and at the mobile phase of methanol, smaller molecules would elute first.

A methanol/PVA gels system is a reversed-phase system and elution is in general in the order of increasing moleuclar weight. For example, in a methanol/polystyrene (PS) gels system (reversed-phase), the elution of phthalate esters and ketones was in the order of increasing molecular weight and in a n-hexane/PS gels system (normal-phase), the elution of them was in the order of decreasing molecular weight[2]. In a chloroform-n-hexane/PS gels for SEC system, the elution of phthalate esters and ketones was in the order of decreasing molecular weight and the increase of resolution was observed with increasing n-hexane in chloroform[4]. In general, a concept that larger molecular weight compounds elute first in normal-phase chromatography in the case of low molecular weight compounds.

However, in the cases of oligomers, solubility of oligomers in solvents used as mobile phases must be considered. Chromatograms for epoxy resins are shown in Figures 2 and 3. Elution of epoxy resins at mobile phase of chloroform was observed to be in the reverse order in comparison with SEC chromatograms obtained in a tetrahydrofuran/PS gels system[5]. Smaller solutes eluted earlier in contrast with other chromatograms. Minimum retention volumes for epoxy resins were obtained at the mobile phase of chloroform/methanol, 80/20 (v/v), but the most effctive size exclusion was attained at the mobile phase of chloroform/methanol, 60/40 (v/v), in comparison with SEC chromatograms[5]. The solubility parameter of the mobile phase of chloroform/methanol, 60/40 (v/v) is 22.9 and that of epoxy resin is 22.3[3]. Therefore, at the mobile phases of chloroform/methanol, 80/20 and 60/40, separation was mainly performed by size exclusion and elution was in the order of decreasing molelcular weight. Larger solutes eluted

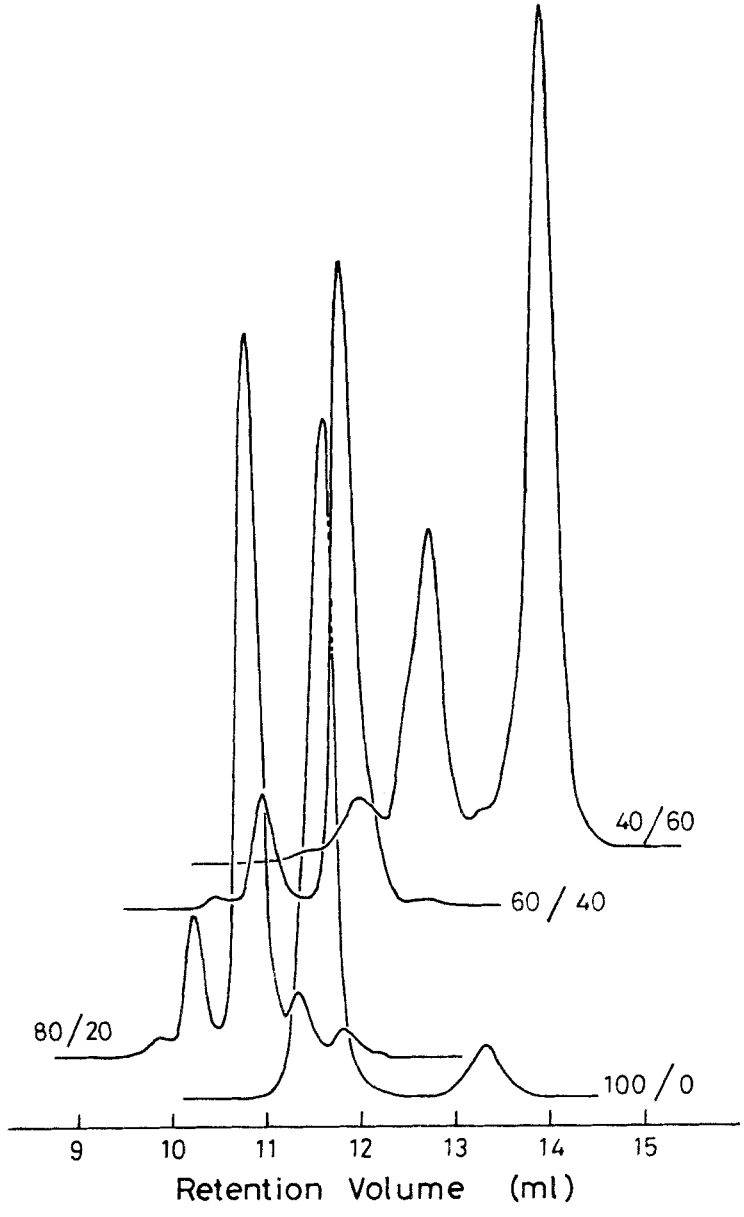

FIGURE 2. Chromatograms of epoxy resin EPIKOTE 828. Composition of mobile phases is shown near each chromatogram as chloroform/methanol (v/v).

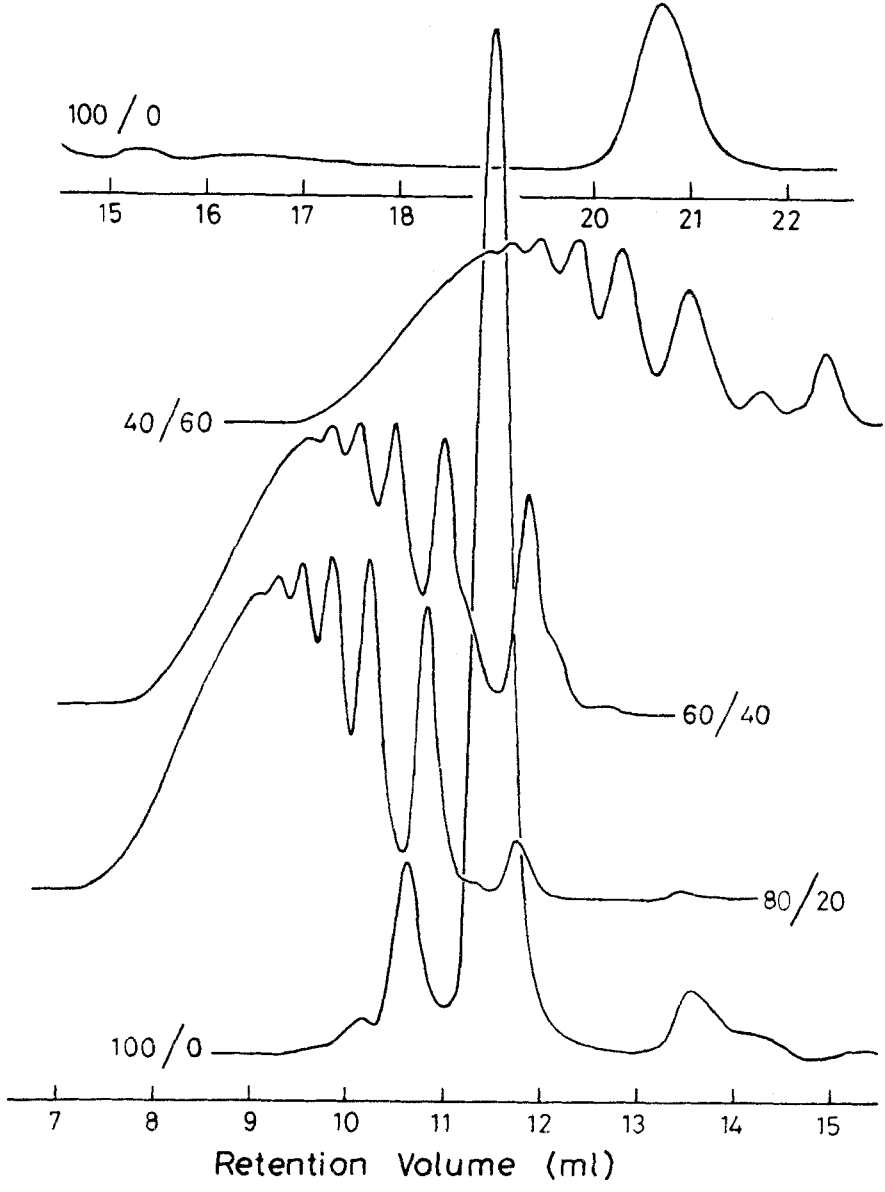

FIGURE 3. Chromatograms of epoxy resin EPIKOTE 1001. Composition of mobile phases is shown near each chromatogram as chloroform/methanol (v/v).

earlier. At mobile phase of chloroform/methanol, 40/60 (v/v), adsorption effects were superimposed on size exclusion.

An epoxy resin/chloroform/PVA gel system corresponds to normal-phase chromatography and smaller solutes eluted first. The solubility parameter of the mobile phase, chloroform/methanol, 40/60 (v/v) is 25.0 (solubility parameter of methanol 29.1) and reversed phase chromatography may be considered in this mobile phase. Elution of epoxy resins in this case was in the order of decreasing moleuclar weight and larger solutes eluted first. These are revese phenomena to those for low-molecular weight compounds as discussed previously. (a methanol/PVA gels/oligostyrene system might be exception).

Figure 4 shows chromatograms of methylated melamine-formaldehyde resin prepolymer. Minimum retention volume was obtained with mobile phase of chloroform/methanol, 80/20 and the shape of the chromatogram was similar to the SEC chromatogram[5]. The composition of this resin prepolymer is complicated and separability was better when mobile phase was chloroform/methanol, 20/80 (v/v). Ten peaks can be observed. The chromatogram obtained with mobile phase of chloroform/methanol, 20/80 (v/v) was divided into four fractions as indicated in Figure 4 and fractions were subjected to HPLC with chloroform as mobile phase. Chromatograms of these fractions are shown in Figure 5. Fraction 4 corresponds to peaks appeared around retention volume between 11.5 and 12.5 ml and fraction 3 coincides with a peak at retention volume 11.0 ml. Fraction 2 covers most of the original peaks except most part of fraction 4 and some part of fraction 3. Therefore, it can be said that separation in this system is mainly by size exclusion and with increasing methanol content in the mobile phase, adsorption effects superimpose on size exclusion. The systems of the mobile phase/PVA gels are reversed-phase and larger molecules eluted first as in the cases of other oligoers. Methylated melamine-formaldehyde resin prepolymers have complicated composition and different structures of same molecular size would appear at the same retention volume by SEC. Therefore, separation with

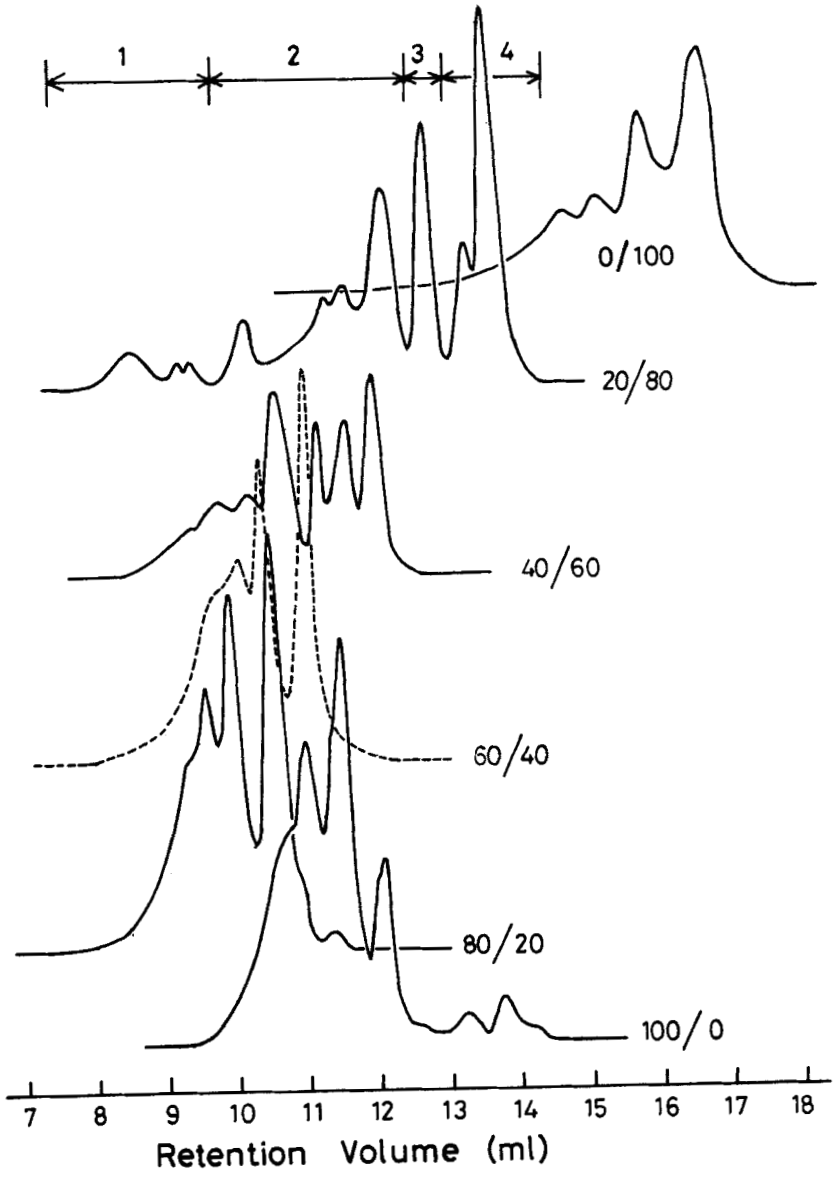

FIGURE 4. Chromatograms of methylated melamine-formaldehyde resin prepolymers. Composition of mobile phases is shown near each chromatogram as chloroform/methanol (v/v).

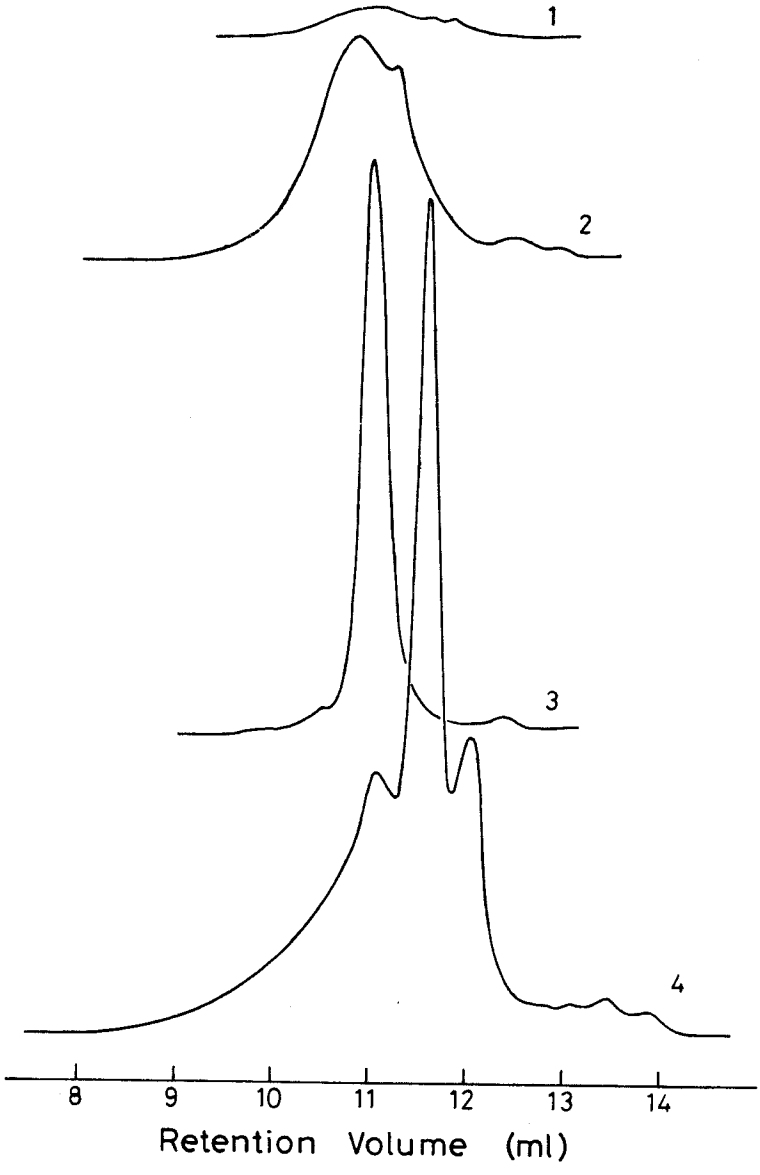

FIGURE 5. Chromatograms of methylated melamine-formaldehyde *resin prepolymers* fractionated with mobile phase of chloroform/methanol, 20/80 (v/v). Mobile phase: chloroform.

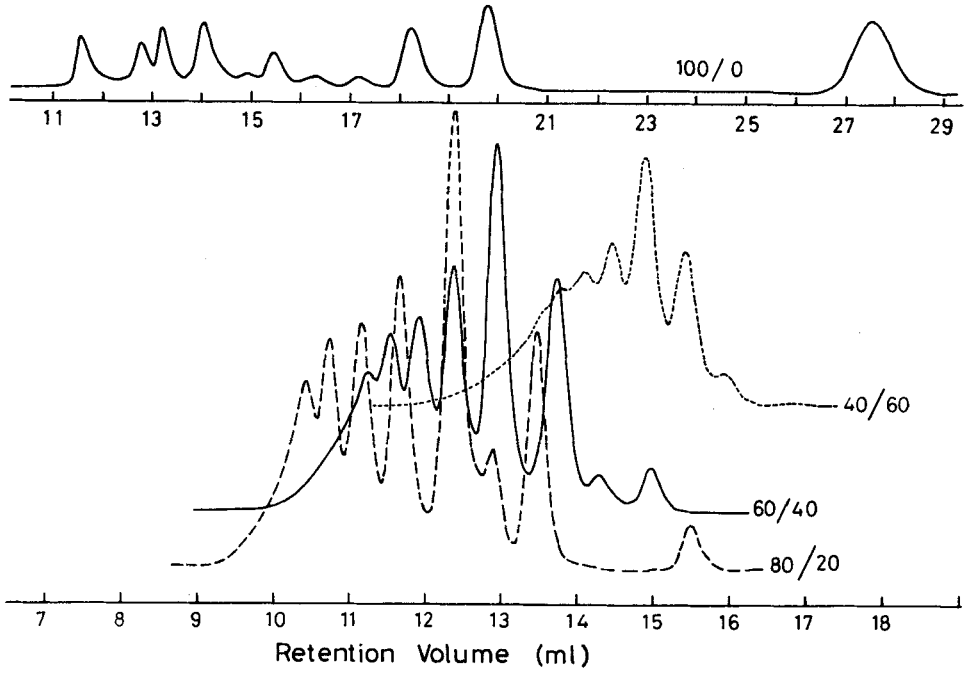

FIGURE 6. Chromatograms of p-cresol-formaldehyde novolac resin prepolymers. Composition of mobile phases is shown near each chromatogram as chloroform/methanol (v/v).

the mobile phase of chloroform/methanol, 20/80 (v/v), followed by fractionation and characterization would give much information.

The minimum retention volume of p-cresol-formaldehyde novolac resin prepolymers shown in Figure 6 was obtained when chloroform/methanol, 80/20 (v/v), was used as mobile phase. The shape of chromatogram is similar to that obtained in SEC of a tetrahydrofuran/PS gels system[5]. Elution of the sample was retarded by increasing methanol content in mobile phase and resolution became worse. It may be clear that adsorption effects were superimposed on size exclusion. Elution would be in the order of decreasing molecular weight. Chloroform might not be a good solvent for SEC

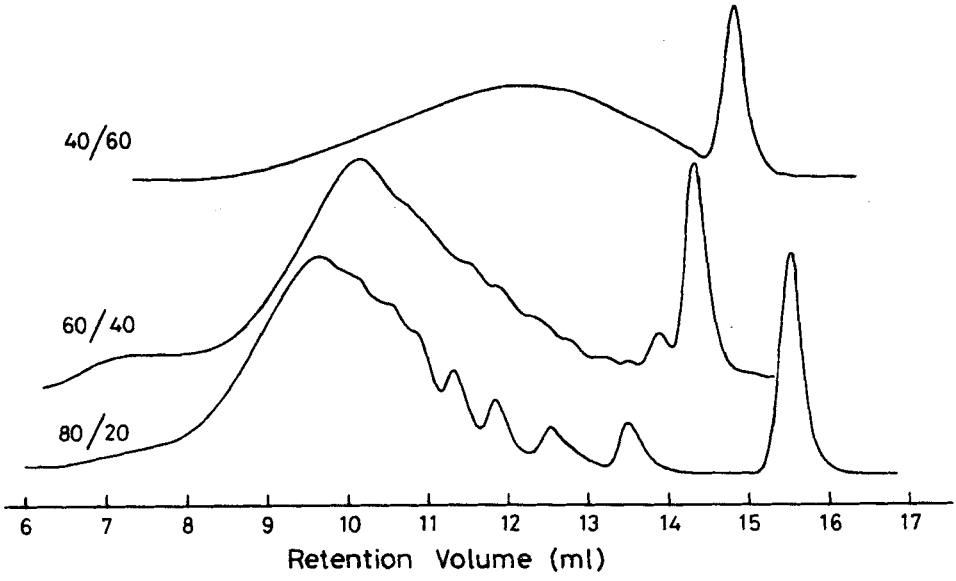

FIGURE 7. Chromatograms of p-cresol-formaldehyde resol resin prepolymers. Composition of mobile phases is shown near each chromatogram as chloroform/methanol (v/v).

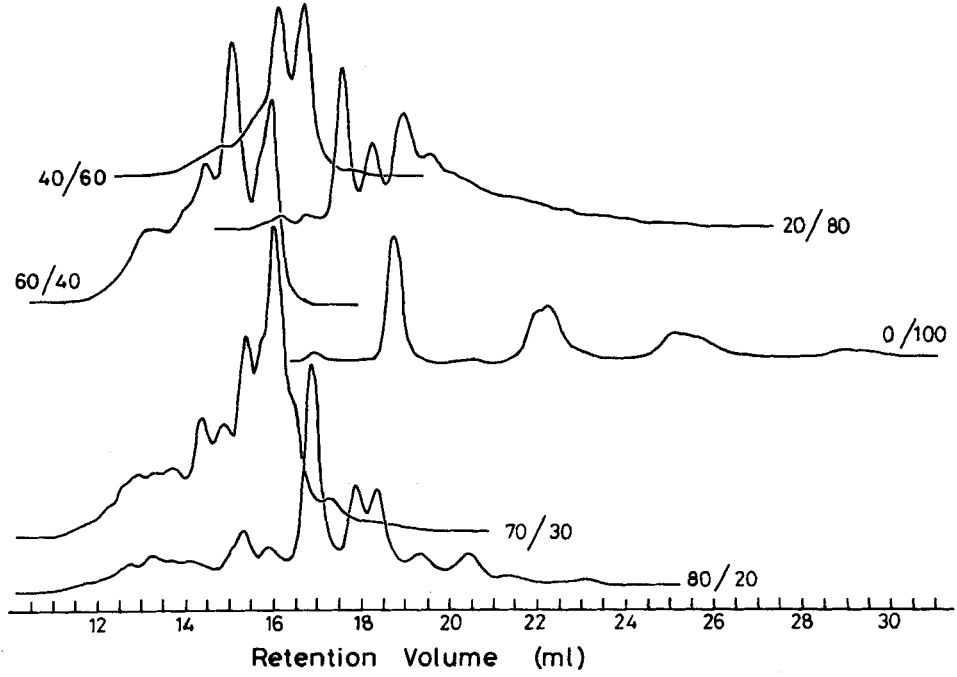

FIGURE 8. Chromatograms of phenol-formaldehyde novolac resin prepolymers. Composition of mobile phases is shown near each chromatogram as chloroform/methanol (v/v).

with PVA gels. A chromatogaram of p-cresol-formaldehyde novolac resin prepolymer obtained with mobile phase of chloroform shows eleven peaks separated completely. The biggest peak appeared at retention volume of 27.5 ml. Characterization of these peaks have not yet made, but p-cresol-formaldehyde novolac resin prepolymers have relatively simple chemical structures and it might be assumed that the elution is in the order of decreasing molecular weight.

Chromaotograms of p-cresol-formaldehyde resol resin prepolymers are shown in Figure 7. The minimum retention volume was obtained with mobile phase of chloroform/methanol, 60/40 (v/v), but resolution was better at chloroform/methanol, 80/20 (v/v).

Figure 8 shows chromatograms of phenol-formaldehyde novolac resin prepolymers. Similar chromatograms to SEC ones [5] were not observed in these samples. Peak shapes were complicated among mobile phases of different composition. Among them, the better resolution was obtained when the mobile phase of chloroform /methanol, 80/20 (v/v) was used. In case of methanol as mobile phase, elution might be in the order of increasing molecular weight.

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