

# Separation and Characterization of Low-Conversion Poly(styrene-co-methyl methacrylate) by Liquid Adsorption Chromatography

SADAO MORI and YOSHITAKA UNO, *Department of Industrial  
Chemistry, Faculty of Engineering, Mie University,  
Tsu, Mie 514, Japan*

## Synopsis

Poly(styrene-co-methyl methacrylate) copolymers of narrow chemical composition distributions were separated according to composition by linear gradient elution at column temperatures between 10 and 80°C using silica gel as an adsorbent. The initial mobile phase was a mixture of chloroform and ethanol (99.0 : 1.0, v/v) and the final one (95.5 : 4.5). The relationship of retention volume and the composition of the copolymers was obtained. Higher column temperature (and/or less ethanol content in the mobile phase) is preferable for the separation of copolymers having less MMA and vice versa. Molecular weight dependence on retention volume was not observed. The chromatographed copolymers were still chemical heterogeneous: the compositional difference of the front and rear halves of an adsorption chromatogram of a copolymer was 2.1%. The front half had a higher styrene content and lower molecular weight averages than those for the rear half of the peak. This separation method was reproducible and the relative standard deviation was about 0.6%. Recovery of fractions was 98.2% by isocratic elution and 96.4% by gradient elution.

## INTRODUCTION

Synthetic random copolymers usually have a chemical composition distribution (CCD) and a molecular weight distribution (MWD). Although MWD of homopolymers can be measured precisely by size exclusion chromatography (SEC), this technique cannot give us accurate information on the MWD of copolymers, because separation in SEC is achieved according to the sizes of molecules in solution; the molecular weights of the copolymers are not necessarily proportional to molecular size. Information on these distributions for copolymers should be obtained by cross-fractionation according to chemical composition and molecular weight.

Several attempts have been reported for the determination of CCD: thin-layer chromatography,<sup>1,2</sup> high-performance liquid chromatography (HPLC),<sup>3-7</sup> orthogonal chromatography,<sup>8</sup> and column adsorption chromatography.<sup>9</sup> Among several techniques to measure CCD, HPLC holds great promise because of its high efficiency. There are several published papers for the separation of copolymers including poly(styrene-co-methyl acrylate)<sup>3</sup> and poly(styrene-co-methyl methacrylate)<sup>4,5</sup> on a silica gel column; poly(styrene-co-acrylonitrile) by precipitation LC on a silica-ODS column;<sup>6</sup> and poly(styrene-co-butadiene) on a polyacrylonitrile gel column.<sup>7</sup>

In our previous papers,<sup>10,11</sup> separation of low-conversion styrene-methyl methacrylate random copolymers, P(S-co-MMA), according to chemical composition by liquid adsorption chromatography (LAC) was reported. The copolymers having more styrene eluted earlier. Ethanol content in the mobile phase (a mixture of chloroform and small amount of ethanol) and column temperature affected the elution of the copolymers. The copolymers having more methyl methacrylate (MMA) tended to elute at a lower column temperature and/or higher ethanol content in the mobile phase.

In the present work, the relationship of retention volume  $V_R$  and the composition of the copolymers measured by changing ethanol content in the mobile phase under the condition of linear gradient elution and column temperature was obtained. The molecular weight dependence of  $V_R$  and the chemical heterogeneity of the copolymers, which are supposed to be relatively uniform in composition, were examined. Reproducibility of this technique was also discussed.

### EXPERIMENTAL

A Jasco TRIROTAR-VI high-performance liquid chromatograph (Jasco Ltd., Hachioji, Tokyo 192, Japan) with an ultraviolet absorption detector (Model UVIDEC-100 VI) at a wavelength of 254 nm and a differential refractive index detector (Model SE-11) (Showa Denko Co., Ltd., Minato-ku, Tokyo 105, Japan) was used throughout this work. The equipment for carrying out gradient elution was a type of low-pressure-mixing gradient system with three solvents. The column used for liquid adsorption chromatography was 50 mm in length and 4.6 mm i.d. and was packed with microporous silica of 30 Å pore size and a mean particle diameter of 5 μm (Nomura Chemical Co., Seto 489, Japan). This column was thermostatted at a specified temperature to a precision of 0.1°C by using a column jacket in which constant temperature water was circulated. The number of theoretical plates  $N$  of the column was 1600 as determined by injecting 5 μL of a 0.5% benzene solution at a flow rate of 0.5 mL/min using chloroform as the mobile phase.

SEC columns are two Shodex A 80 M HPSEC columns (50 cm × 8 mm i.d.) (Showa Denko Co.) packed with a mixture of polystyrene gels of nominal exclusion limits of 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup>, and 10<sup>6</sup> Å. The efficiency was 17,000 plates per 50 cm, which was obtained by injecting 0.1 mL of a 0.1% benzene solution at a flow rate of 1.0 mL/min.

P(S-MMA) samples were prepared by solution polymerization at a low degree of conversion. The composition of the samples was measured by ultraviolet spectrophotometry and were found to have a styrene content as follows: copolymer I(85.5%), II(73.4%), III(66.3%), IV(57.4%), V(48.7%), VI(42.1%), VII(41.5%), VIII(26.5%), and IX(15.2%). The samples were dissolved in chloroform and absorbance at 260 nm was measured and then the composition was calculated from a calibration curve which was constructed from polystyrene.

The mobile phase for LAC was a mixture of chloroform and ethanol and the composition of the mobile phase was regulated by linear gradient elution. The flow rate was 0.5 mL/min. Ethanol used as a stabilizer in chloroform was removed before use by extracting the chloroform with water. Henceforth,

chloroform in this report means that it does not contain any ethanol unless otherwise specified. The initial mobile phase (A) for linear gradient elution was a mixture of chloroform and ethanol (99.0 : 1.0, v/v) and the composition of the final mobile phase (B) was 95.5 : 4.5 (v/v) chloroform/ethanol. The composition of the mobile phase was changed from 100% A to 100% B in 15 min and kept to 100% B for another 10 min. Samples were dissolved in A in a concentration of 0.05% and a portion of 0.05 mL of the sample solution was injected 1 min after from the start of gradient. The mobile phase for SEC was chloroform containing 1% ethanol and the flow rate was 1 mL/min.

The composition of copolymers in LAC and SEC fractions was measured by infrared spectrophotometry. Fractions were dried on a KBr disk and film formed on the disk was subjected to the measurement of infrared spectra. The absorbance ratio of  $1728\text{ cm}^{-1}$  (a C = O characteristic band) to  $698\text{ cm}^{-1}$  (a phenyl characteristic band) was determined and the composition of a styrene content was calculated by using a calibration curve which was constructed with the copolymer samples of known composition by a similar method. The absorbance ratio was plotted as ordinate and MMA content as abscissa. Styrene content was obtained as difference.

## RESULTS AND DISCUSSION

### Retention Volume vs. Copolymer Composition

In our paper,<sup>11</sup> it was reported that copolymers having more MMA tended to elute from the column with increasing ethanol content in the mobile phase under isocratic elution conditions. Only polystyrene eluted from the column

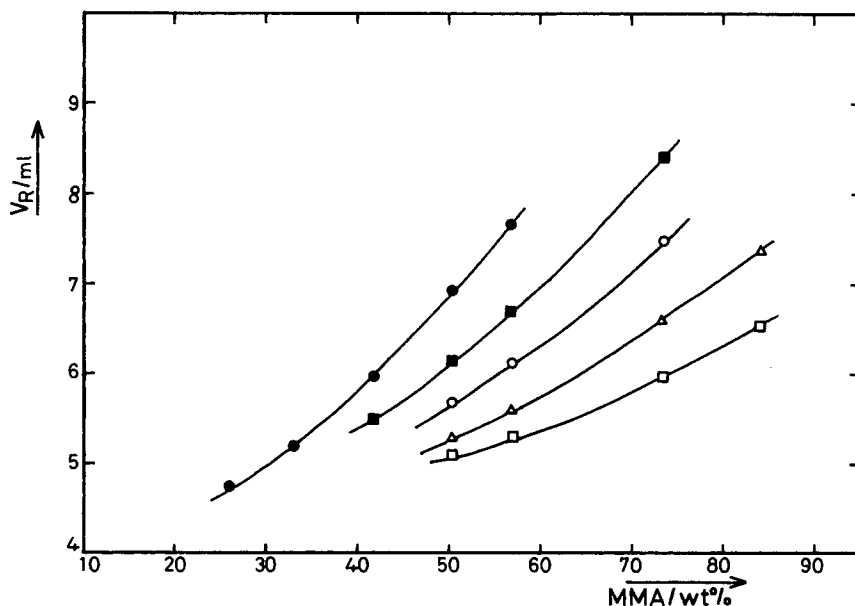


Fig. 1. Relationship of retention volume vs. MMA content of P(S-MMA) at different column temperature. (●) 80°C; (■) 60°C; (○) 50°C; (△) 40°C; (□) 30°C. Sample concentration: 0.05%; injection volume: 0.05 mL. For conditions of gradient elution see Experimental section.

with pure chloroform as the mobile phase and all the copolymers were retained in the column. At a column temperature of 10°C, copolymer I eluted from the column with the mobile phase containing 0.5% ethanol in chloroform. Similarly, copolymers I to IV appeared from the column at 1.0% ethanol concentration in chloroform, copolymers I to VI at 1.5% ethanol in chloroform, copolymers I to VIII at 2.0% ethanol in chloroform, and all the copolymers at 2.5% ethanol in chloroform.

Column temperature also influenced the elution of the copolymers. When the chloroform/ethanol mobile phase composition was (99.0:1.0,v/v) copolymer I eluted from the column at column temperatures between 10 and 80°C and copolymer II eluted from the column at column temperatures between 10 and 60°C. Similarly, copolymer III adsorbed in the column at over 35°C and copolymer IV at over 20°C. Other copolymers did not elute from the column at all even at a column temperature of 10°C. However, ethanol content or column temperature did not affect peak retention volume for the copolymers: when they did elute from the column,  $V_R$  was always at the interstitial volume  $V_0$  and no adsorption was observed.

Therefore, to change  $V_R$  of copolymers of different composition, linear gradient elution was used. Figure 1 shows the relationship between peak retention volume and MMA content for the copolymers obtained at column temperatures between 30 and 80°C. (Linear gradient elution conditions are given in the Experimental section.) Copolymer IX eluted from the column at column temperatures 30 and 40°C, but was retained at over 50°C. Copolymers VIII and IX did not elute from the column at 80°C. Peak resolution decreased

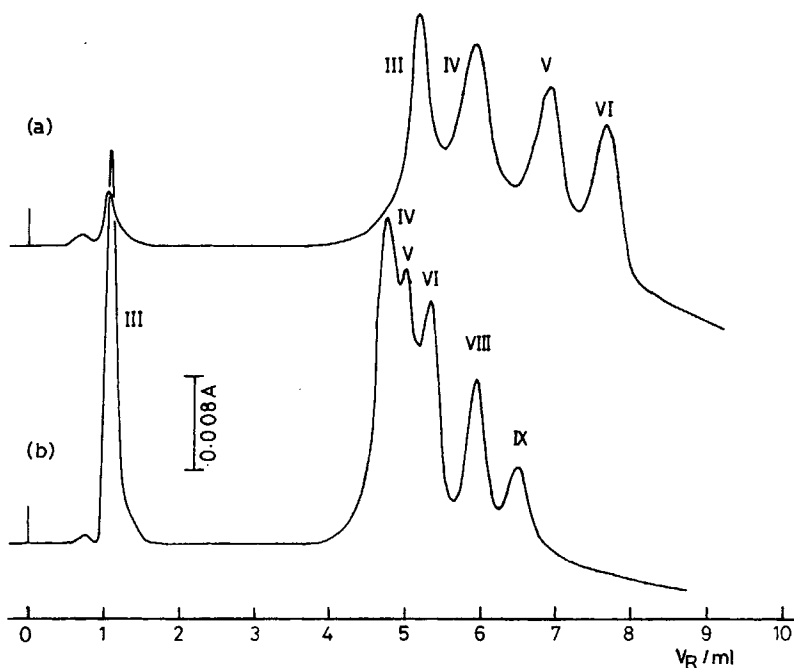


Fig. 2. LAC chromatograms of P(S-MMA). Column temperature: (a) 80°C, (b) 30°C; sample concentration: 0.05% each; UV attenuation: 0.08AUFS. For other conditions see text.

with decreasing column temperature. Thus higher column temperature (and/or less ethanol content in the mobile phase) is preferable for the separation of copolymers having less MMA and more ethanol content in the mobile phase (and/or lower column temperature) for those having more MMA.

Examples for the separation of a mixture of copolymers III, IV, V, VI, VIII, and IX are shown in Figure 2. (Conditions for gradient elution are the same as in Figure 1.) Figure 2(a) was obtained at a column temperature of 80°C and copolymers VIII and IX were retained in the column. Copolymer I, if any, might appear at interstitial volume (in this case  $V_R = 1$  mL). By lowering the column temperature to 30°C, copolymers VIII and IX eluted from the column, but copolymer III appeared at interstitial volume. Moreover, copolymers IV, V, and VI were not separated well. The gap between peaks III and IV is defined as a blind zone in which no peak appears as discussed later.

### Molecular Weight Dependence of Retention Volume

An elution chromatogram of copolymer III is shown in Figure 3(a). In order to determine the molecular weight dependence of peak width and retention volume, this copolymer was fractionated by SEC into four fractions. LAC chromatograms of these fractions are shown in Figure 3(b)–(e). Characteristics of these fractions are listed in Table I.

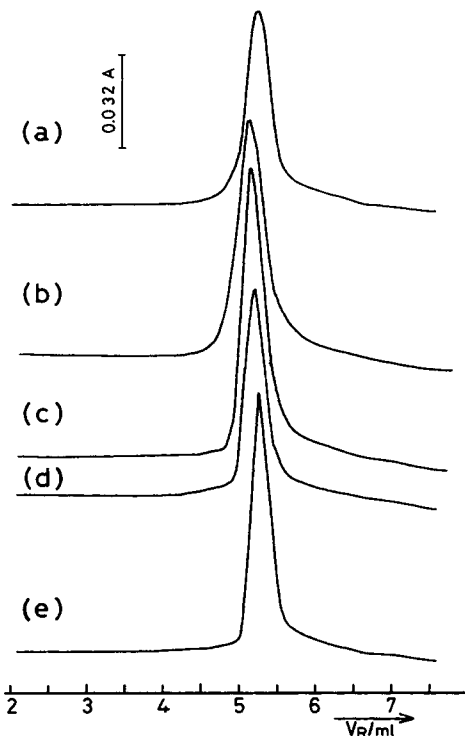


Fig. 3. LAC chromatograms of copolymer III unfractionated (a) and fractionated by SEC (b–e). (b) Fraction 4; (c) fraction 3; (d) fraction 2; (e) fraction 1. Column temperature: 80°C. For other conditions see text.

TABLE I  
Characteristics of SEC Fractions of Copolymer III

Fraction no.	$V_R$ in SEC (mL)	Composition <sup>a</sup> st (wt%)	Molecular weight		Peak width at half height (mm)
			$M_w \times 10^{-5}$	$M_n \times 10^{-5}$	
1	22.6-26.2	67.1	2.35	2.15	5.3
2	26.2-27.2	67.1	1.27	1.25	6.2
3	27.2-28.5	68.4	0.82	0.80	6.8
4	28.5-34.6	70.0	0.39	0.29	8.5
Unfractionated	22.6-34.6	67.6	1.17	0.65	6.8

<sup>a</sup>Composition was measured by infrared spectrometry.

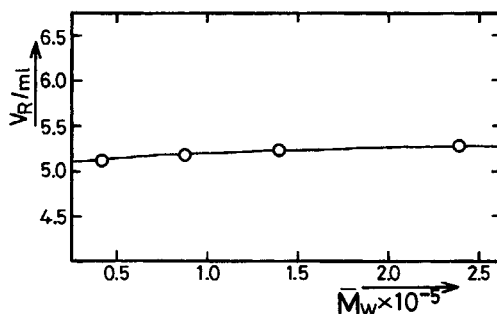


Fig. 4. Relationship between retention volume and molecular weight of copolymer III fractions constructed from Figure 3.

Peak retention volume of each fraction is almost the same, signifying negligible molecular weight dependence. The relationship between retention volume and molecular weight of copolymer III is shown in Figure 4. Small difference in retention volume is probably caused by composition differences. However, peak width of fractions increased with decreasing molecular weight, especially for Fraction 4; the reason for this is not clear. CCD and MWD of fractions are smaller than unfractionated ones. Considering the results of the next section, peak width at half height might be to some extent influenced by molecular weight.

### Chemical Heterogeneity of Samples

LAC chromatogram of copolymer III was divided into two fractions, front and rear halves, as shown in Figure 5. The concentration of the unfractionated sample was 0.06% and injection volume was 0.1 mL. Fractionation was repeated 50 times and each fraction was combined. Each combined fraction was evaporated and redissolved in chloroform into 5 mL (0.03% concentration). The front half eluted earlier ( $\Delta V_R = 0.11$  mL), contained more styrene, and had lower molecular weight. However, peak width at half height of each fraction was the same as the unfractionated peak. Characteristics of the fractions are listed in Table II. The fraction having more MMA had higher molecular weight, which is similar to the results found in previous studies.<sup>10,11</sup>

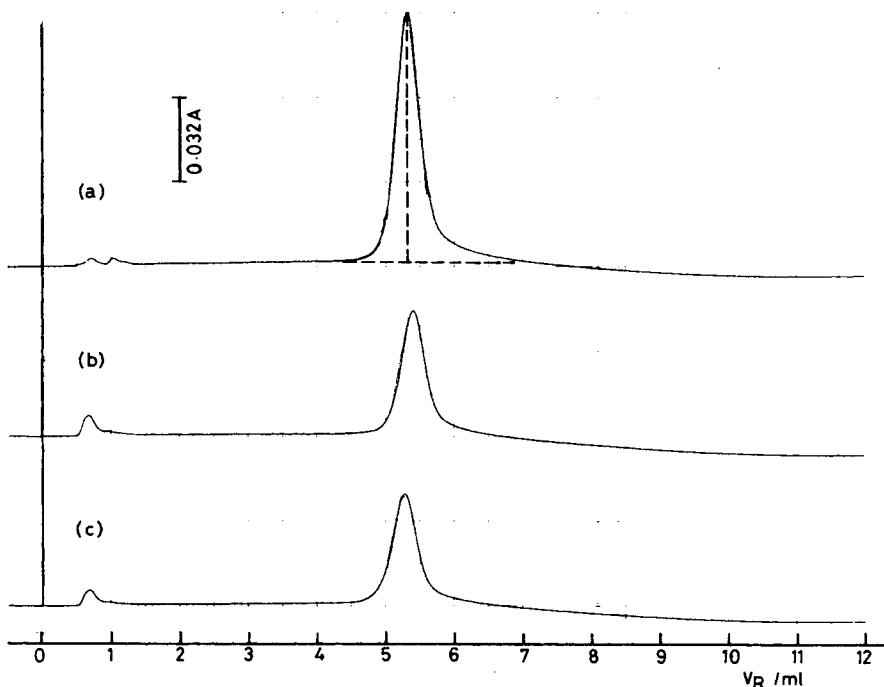


Fig. 5. LAC chromatograms of fractions of copolymer III fractionated by LAC. (a) Unfractionated copolymer III; (b) rear half of copolymer III fractions; (c) front half of copolymer III fractions.

Composition of sample copolymers was measured by ultraviolet (UV) spectrophotometry, because a UV method is more convenient and easier to use for measurement than an IR method. The relative standard deviation of a UV method was between 0.1 and 0.6% depending on styrene content. However, concentration of the copolymers in fractions was not clear and composition of copolymers in LAC and SEC fractions had to be measured by an IR method as described in the Experimental section. The relative standard deviation of an IR method was between 0.3 and 0.6%. Therefore, the difference in the two results in Table II is regarded as significant. The difference of a styrene content in Table I is also regarded as significant.

TABLE II  
Characteristics of LAC Fractions of Copolymer III

Fraction	Composition <sup>a</sup> st (wt%)	Molecular weight	
		$\bar{M}_w \times 10^{-5}$	$\bar{M}_n \times 10^{-5}$
Front half	68.3	1.08	0.56
Rear half	66.2	1.29	0.76
Unfractionated	67.6	1.17	0.65

<sup>a</sup> Measured by IR.

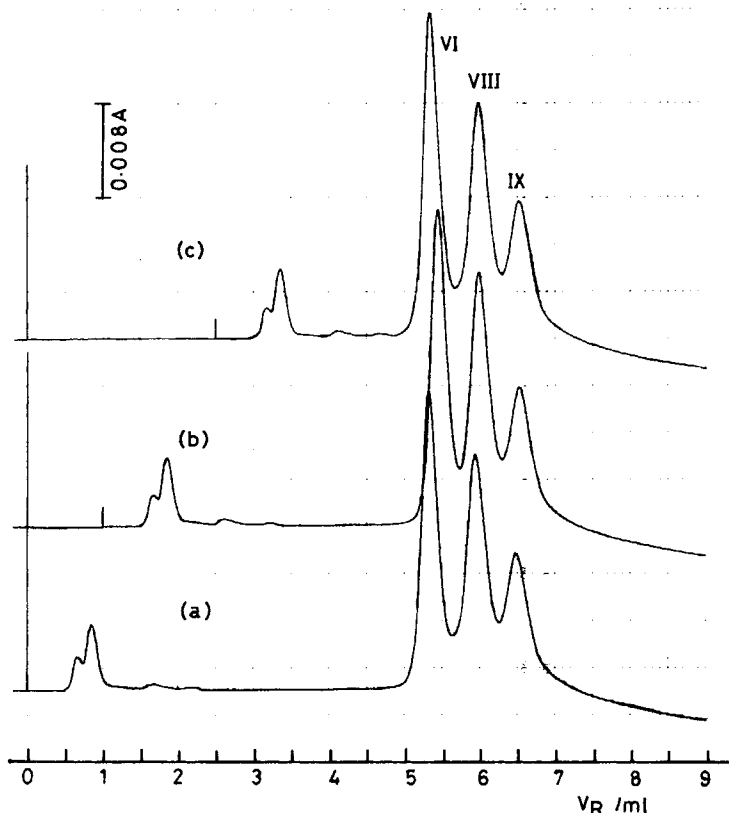


Fig. 6. Gradient elution chromatograms of copolymers VI, VIII, and IX at different sample injection time. (a) Injection at the start of gradient elution; (b) injection after 2 min; (c) 5 min; (d) 6 min; (e) 7 min from the start of gradient elution. Column temperature: 30°C; sample concentration: 0.5% each; injection volume: 0.1 mL.

### Reproducibility and Repeatability

Figure 6 shows the LAC chromatograms of a mixture of copolymers VI, VIII, and IX obtained at different injection times after the start of gradient elution. Retention volume of each peak was measured from the starting point of gradient elution. The results are listed in Table III.

Retention volume of each copolymer is almost constant regardless of the different sample injection time from the start of gradient elution. This result means that once adsorbed on silica gel surface, the sample does not desorb from the surface until the composition of the mobile phase reaches a specified value which is defined as a critical composition of the mobile phase. Ethanol content in the mobile phase at  $V_R$  5.4 mL is 3.4% according to the gradient programmer. However, the time that the mobile phase of the specified composition arrives at the column from the gradient generator (a mixer) is about 5 min ( $V_R$  2.5 mL) and this delay time must be compensated for. Hence, the calculated ethanol content in the mobile phase at  $V_R$  5.4 mL is about 2.3%.

When gradient elution was performed, two mobile phases A and B were mixed in a small chamber which was placed between solvent reservoirs and a



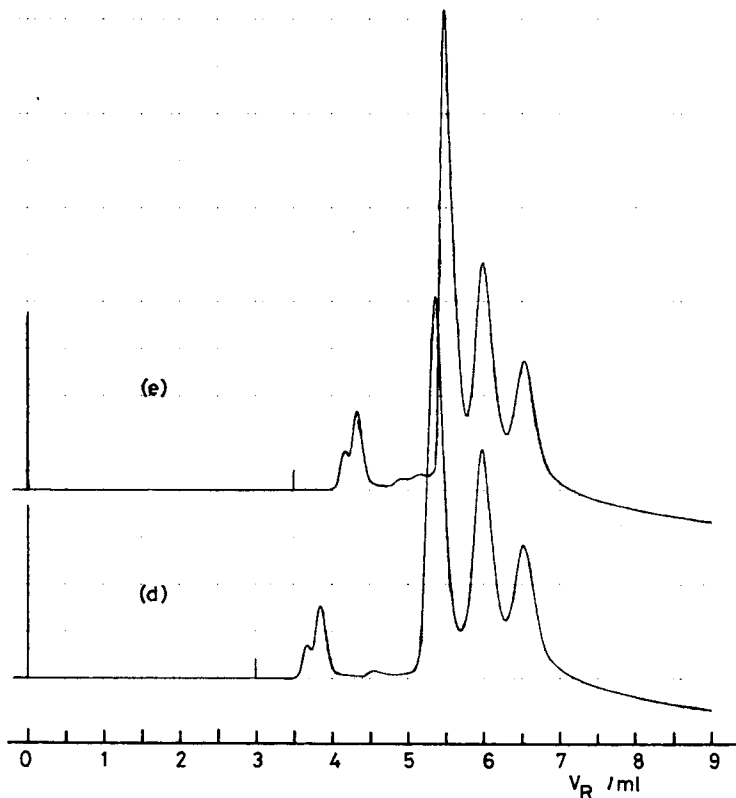


Fig. 6. (Continued from the previous page.)

pump. Hence, the actual ethanol content in the mobile phase might be much lower than calculated. Therefore, the copolymers are adsorbed on the surface of silica gel up to  $V_R$  5 mL and the retention volumes remain unchanged regardless of the different sample injection time. Furthermore, the unchanged retention volumes show that this method is reproducible. If this sample solution were injected 13 min after the start of the gradient, then all the copolymers would appear at the interstitial volume  $V_0$ , because no adsorption would occur on the surface of silica gel in advance. Figure 6 ends at  $V_R$  9 mL,

TABLE III  
Retention Time of Copolymers VI, VIII, and IX at Different Injection Time

Injection Time <sup>a</sup> (min)	Retention time <sup>b</sup>		
	VI	VIII	IX
0	10.66	11.90	12.96
2	10.88	11.98	13.05
5	10.68	11.96	13.05
6	10.72	11.96	13.05
7	10.96	11.98	13.06

<sup>a</sup> Time elapsed after starting gradient elution.

<sup>b</sup> Time from the start of gradient elution.

TABLE IV  
 Recovery of Copolymers V, VI, and VIII

Sample	Peak area			Recovery <sup>c</sup>	
	Theoretical <sup>a</sup>	Isocratic <sup>b</sup>	Gradient <sup>b</sup>	Isocratic	Gradient
V	2.95	2.85	2.85	96.6	96.6
VI	2.56	2.46	2.53	96.1	98.8
VIII	1.62	1.65	1.52	101.9	93.8

<sup>a</sup>(Polystyrene peak area) × (Styrene weight fraction of copolymer).

<sup>b</sup>Column temperature: 30°C.

<sup>c</sup>Recovery =  $\frac{\text{area by isocratic or gradient elution}}{\text{theoretical area}} \times 100$ .

and a baseline still declines somewhat. Actually, it became level at  $V_R$  11 mL and the difference of UV absorbance between  $V_R$  9 and 11 mL was 0.0016 absorbance units.

In Figure 2, copolymers eluted at  $V_0$  at a mobile phase composition where copolymers do not adsorb on the surface of silica gel. For example, copolymers I, II, and III eluted at  $V_0$  when the initial composition of the mobile phase was 99.0:1.0 (v/v) chloroform/ethanol at a column temperature of 30°C. Therefore, copolymer II eluted at  $V_0$  as in Figure 2(b). Other copolymers started to elute with increasing ethanol content in the mobile phase and copolymer IV eluted at  $V_R$  4.7 mL, where the calculated ethanol content was about 2.0%. A space between  $V_0$  and the point where copolymer IV eluted can be defined as a blind zone in which no sample elutes.

The retention volume repeatability of this method was examined by injecting the mixture of copolymers VI, VIII, and IX into the column 20 times. The relative standard deviation of the retention volumes was between 0.58 and 0.64%. Recovery of copolymers injected into the column was also examined and the results are summarized in Table IV. Recovery was calculated from peak areas of copolymers V, VI, and VIII. Theoretical peak area was assumed to be that of the peak area of polystyrene of the same weight multiplied by the styrene fraction of the copolymer. Percent recovery was also calculated as the quotient of the experimental peak area of a copolymer divided by the theoretical peak area. Sample VIII, using the isocratic mode showed high recovery which probably was due to experimental error, owing to its small peak size as compared with other samples.

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