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CONCENTRATION EFFECT IN GEL PERMEATION CHROMATOGRAPHY

II. VISCOSITY PHENOMENA IN THE INTERSTITIAL VOLUME

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SUMMARY

The effect of the viscosity of the injected polymer solution in gel permeation chromatography was investigated using samples of polystyrene standards with molecular weights above the exclusion limit of the column packing used. It was confirmed that a linear relationship existed between the specific viscosity of the injected polymer solution and the elution volume in the range of the flow-rates in which there was no essential effect of non-Newtonian flow. At higher flow-rates of the solvent, non-Newtonian behaviour of the polymer solution in the chromatographic column was observed.

INTRODUCTION

In Part I of this series[1], the dependence of the elution volume on the concentration of the injected polymer solution in gel permeation chromatography (GPC) on porous glass was studied, under conditions such that the size of the accessible pores did not change with changing thermodynamic properties of the solvent. Equations were derived that quantitatively described the processes responsible for this concentration dependence. It was assumed that the viscosity effect in the interstitial volume also contributed to the overall concentration effect. This assumption was expressed quantitatively as follows:

$$V_v = \frac{k'}{L} \cdot \int_0^L \eta_{spec} \cdot du \qquad (1)$$

V_v is the elution volume increment proportional to the difference in the viscosities of the polymer solution along the column, η_{spec} is the specific viscosity of the polymer solution varying during the movement of the chromatographic zone through the column, L is the column length, k' is a proportionality constant and u is the axial coordinate of the chromatographic column. The work described here is an attempt to verify experimentally the general validity of the above assumption.

EXPERIMENTAL

Gel permeation chromatography

GPC measurements were performed with a purpose-built apparatus. A linear feeder of the type usual in the continuous feeding of liquids into chemical reactors was used as the solvent pump. The linear rate of movement of the piston, and thus also the flow-rate of the solvent, were constant with an accuracy greater than 0.05% (accuracy of the experimental method) at all flow-rates used. The measurement of the elution volume was derived from the movement of mechanical parts of the linear feeder and was therefore independent of the flow-rate. The volume of one count was 0.2091 ml in all instances.

The samples were injected using a six-port injection valve (Waters Assoc., Milford, Mass., U.S.A.) and a loop, the total volume injected being 80 μl. The separation column was 30 cm long, 8 mm I.D., and the standard end fitting of the column (Waters) had concentric radial grooves providing a uniform flow distribution throughout the cross-section at the beginning of the column. The stainless-steel fritted discs were replaced with simple stainless-steel sieves of aperture diameter 40 μm. The column was placed in a jacket thermostated, together with the refractometer, at $25 \pm 0.01°$. The R-403 differential refractometer (Waters) was adjusted by reducing the inner diameter of the inlet capillary to 0.25 mm, so that the overall dead volume was reduced as much as possible (to *ca.* 60 μl). The reference cell of the refractometer was connected in the hydraulic circuit between the pump and the injection valve, which was attached to the head of the column with a capillary of I.D. 1 mm and length 5 cm. The silica gel column packing (Porasil B, Waters) was sieved to a particle size of 63–71 μm. A visual microscopical check of the sieving confirmed that there were no irregular or dust particles in the silica gel after sieving. The column was packed in the dry state by the gradual addition of silica gel and tapping. Tetrahydrofuran (THF), distilled from copper(I) chloride, was used as a solvent.

Polystyrene samples

Polystyrene (PS) standards (Waters) with a narrow molecular weight distribution ($\bar{M}_w/\bar{M}_n < 1.1$) were used. The designation of the PS standards and the molecular parameters are given in Table I. All calculations were carried out using the Mark–Houwink equation:

$$[\eta] = 1.17 \cdot 10^{-2} M^{0.717} \tag{2}$$

valid for linear PS in THF at 25°, and the Huggins constant $k_H = 0.362$; the relevant literature references were given in Part I[1].

RESULTS AND DISCUSSION

In reproducing experiments described in Part I[1], but using a column of a higher efficiency as characterized by the height equivalent to a theoretical plate (HETP), we obtained a different shape of the gel permeation chromatograms. It was obvious that some important phenomena could be suppressed owing to a greater dispersion of the chromatographic zone. It was our aim to reduce the total dispersion

of the chromatographic zone as much as possible, which was achieved by careful sieving of the packing, high-quality packing, adjustment of the end fittings of the column and choosing a smaller length of the column.

The calibration graph presented in Fig. 1 was obtained by measurements on PS standards, the concentration of the injected solutions being 0.05% (w/v). Fig. 1 shows that the PS standards with a molecular weight higher than 50 000 lie above the exclusion limit of the packing used and consequently their molecules migrate in the interstitial volume only.

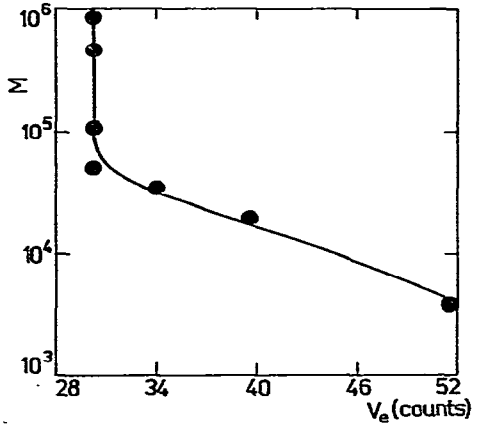


Fig. 1. Calibration graph for the chromatographic column used.

Further investigations were carried out solely with the standards PS 1–PS 3, the molecular weights of which lay above the exclusion limit. The concentrations of these standards were chosen so as to make the η_{spec} values of solutions of different standards always the same, within the limits of experimental error. The η_{spec} values and the corresponding concentrations calculated for the individual standards PS 1–PS 3 using the Huggins equation, the Mark–Houwink equation and the constant k_H are given in Table I. We prepared solutions of the standards PS 1–PS 3 having the highest concentrations given in Table I, lower concentrations being were obtained by diluting the solutions.

TABLE I

MOLECULAR WEIGHTS OF PS STANDARDS AND CONCENTRATIONS AT GIVEN η_{spec} VALUES

Standard	*Molecular weight*	*Concentration of PS standards (%, w/v) at nine η_{spec} values*								
	$\bar{M}_w \cdot 10^{-3}$	*7.286*	*2.650*	*1.077*	*0.476*	*0.223*	*0.107*	*0.053*	*0.026*	*0.013*
PS 1	2610	0.71	0.355	0.1775	0.0888	0.0444	0.0222	0.0111		
PS 2	867		0.783	0.3915	0.1958	0.0979	0.0489	0.0245	0.0122	
PS 3	470		1.214	0.607	0.3035	0.1518	0.0759	0.0379	0.0190	0.0095
PS 4	110									
PS 5	51									
PS 6	35									
PS 7	20.8									
PS 8	3.6									

Chromatograms of the standard PS 1 at different injected concentrations and the same sensitivity of the refractometer at a flow-rate of 0.334 ml/min are shown in Fig. 2. Chromatogram 1 was obtained with an injected solution of the standard PS 1 having the lowest η_{spec} (0.053); chromatogram 7 was recorded with an injected solution with the highest η_{spec} (7.286). Chromatograms 1–4, taken at low η_{spec} values, are very similar in character. Chromatograms 5–7, at higher η_{spec} values, possess a more complicated structure; in all instances there is a distinct increase in the width of the chromatograms, and the elution volumes are shifted to higher values. Similar observations were made by Moore[2], who attributed them to viscous fingering[3].

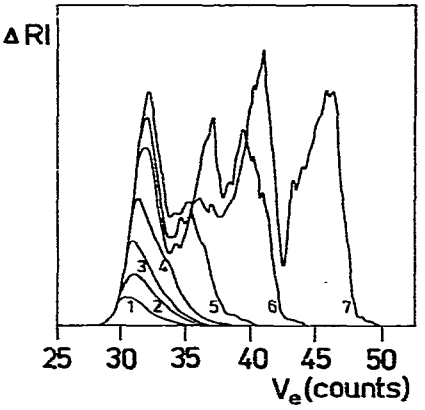


Fig. 2. Chromatograms of standard PS 1 at a flow-rate of 0.334 ml/min. Specific viscosity of injected solution: η_{spec} = 0.053 (chromatogram 1), 0.107 (2), 0.223 (3), 0.476 (4), 1.077 (5), 2.650 (6), 7.286 (7).

We tried to determine the relationship between the average elution volume and the width of the chromatographic zone at various η_{spec} values. Average elution volumes (V_{av}) were calculated from the chromatograms using the expression

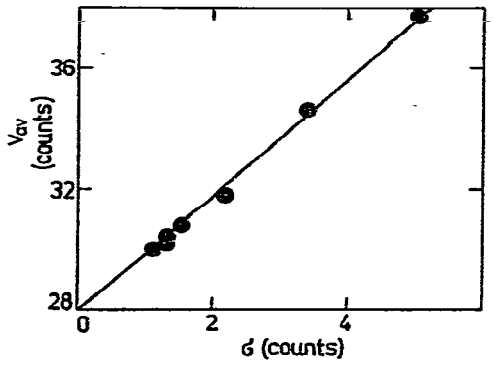
$$V_{av} = \frac{\Sigma V_i \cdot h_i}{\Sigma h_i} \tag{3}$$

where h_i are the heights of the chromatograms from the baseline in the elution volumes V_i. The band width of the elution curves was characterized by the variance, var (V), according to

$$\text{var}\,(V) = \frac{\Sigma(h_i \cdot [V_i - V_{av}]^2)}{\Sigma h_i} \tag{4}$$

where var (V) = σ^2 and σ is the standard deviation. In Fig. 3, σ values are plotted against V_{av} for the individual chromatograms in Fig. 2. As can be seen in Fig. 3, there is a linear relationship between σ and V_{av}. This linear relationship was also observed at other flow-rates (0.038 and 3.0 ml/min).

If the assumption expressed by eqn. 1 is correct, then experiments should show that the elution volumes are the same for different PS standards whose injected solutions have the same η_{spec} values. The experimental results shown in Fig. 4 confirm the correctness of the above assumption. With injected solutions of the standards



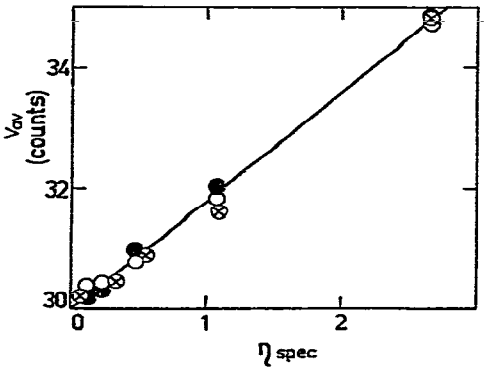


Fig. 3. Dependence of the elution volume (V_{av}) on the width of the chromatogram (σ) (measured at a flow-rate of 0.334 ml/min).

Fig. 4. Dependence of the elution volume (V_{av}) on the specific viscosity (η_{spec}) of solutions of various polystyrene standards: ●, PS 1; ○, PS 2; ⊗, PS 3; flow rate, 0.334 ml/min.

PS 1–PS 3 having the same η_{spec} values, we not only obtained the same V_{av} values, within the limits of experimental error, but the chromatograms for the standards PS 2 and PS 3 were also the same as those of the standard PS 1 in Fig. 2. The linear dependence of V_{av} on η_{spec} within the investigated range of η_{spec} values was also confirmed by the experiment. The results in Fig. 4 were measured at a flow-rate of 0.334 ml/min, but the measurement was also performed at a flow-rate of 0.038 ml/min with similar results.

On extending the range of η_{spec} values of the injected solutions up to 7.286, a deviation from the linearity of the relationship between V_{av} and η_{spec} was observed, which was greater at a flow-rate of 0.334 ml/min than at 0.038 ml/min, as shown in Fig. 5. At a flow-rate of 3 ml/min, the deviation from linearity was even more pronounced; moreover, it began at lower η_{spec} values (Fig. 5). These deviations from linearity may be explained by the non-Newtonian behaviour of more viscous solutions

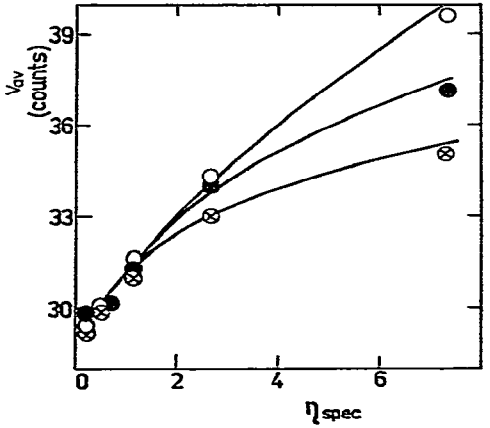


Fig. 5. Dependence of the elution volume (V_{av}) on the specific viscosity (η_{spec}) at various flow-rates: ○, 0.038; ●, 0.334; ⊗, 3.0 ml/min.

of the injected PS standards. The viscosity of concentrated solutions depends on tangential stress[4], which is related to the trans-channel velocity gradient[5]. The decrease in the effect of concentration on the GPC results at high flow-rates (10–35 ml/min) observed by Little *et al.*[6,7] can probably be explained in this way.

The results reported here have confirmed with fairly good accuracy the preceding theoretical reasoning concerning the effect of the viscosity of the polymer solution in GPC column separation. Some other aspects of the viscosity effect and general concentration effects are currently being studied.

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