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CONCENTRATION EFFECTS IN GEL PERMEATION CHROMATOGRAPHY

III. INFLUENCE OF COLUMN EFFICIENCY

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SUMMARY

Investigation of concentration effects in gel permeation chromatography has shown that the results depend on the efficiency of the column. The shape of elution curves, especially those of completely excluded macromolecules, is strongly dependent on the concentration of the injected polymer solution. If a simple procedure of evaluation of experimental data from the elution volumes of the maxima of elution curves and from the graphically determined chromatogram widths is used, the contribution of the viscosity effect to the total change in the elution volume with concentration is greatly underestimated. A good correlation between experimental results and theoretical predictions is obtained if data are evaluated using statistical parameters of the elution curves.

INTRODUCTION

Concentration effects in the gel permeation chromatography (GPC) of polymers using rigid porous column packings may be due to a number of processes that participate to various extents in changes in the elution volume and width of the chromatographic band. In Part I[1], equations were derived that describe quantitatively the concentration dependence of the elution volume, assuming that under the given experimental conditions the effect of the viscosity of the eluted sample solution and the effect of the expansion of macromolecular coils contribute to the overall concentration effect. It has been established that the viscosity effect is about one third of the two contributing effects, but complete accord could not be achieved between the experimentally observed change in the elution volumes following a change in concentration on the one hand, and the calculated change on the other. Using a four-fold shorter column with better efficiency, characterized by the height equivalent to a theoretical plate (HETP), we found[2] that the shape of the elution curves differed greatly from the previous ones[1], particularly at higher concentrations of injected polymer solutions. To characterize the elution volume and the width of very deformed elution curves, statistical parameters had to be used, instead of the previously used elution volume of the maximum and graphical evaluation of the width of the elution curve. It seemed that some important phenomena might be suppressed by a larger dispersion of the chromatographic band on less efficient columns. For this reason, we reproduced some of the experiments reported in Part I on a column with higher efficiency.

The elution volume, V_e, and its dependence on concentration (without viscosity effects) can be calculated by means of the equation

$$V_e = P + Q \ln v + \frac{Q}{L} \int_0^L \ln \varepsilon \, du \tag{1}$$

where P and Q are constants, L is the column length, v is the volume of the unswollen macromolecule, ε is the effective volume factor of swelling (a function of concentration) and u is the axial coordinate of the column. The contribution to the elution volume, V_v, due to the viscosity effect in the interstitial volume is proportional to the difference between the viscosities of the polymer solution and the solvent, and can be calculated from

$$V_v = \frac{k'}{L} \int_0^L \eta_{\mathrm{spec}} \, du \tag{2}$$

where k' is a proportionality constant and η_{spec} is the specific viscosity of the polymer solution, which varies with concentration along the column.

The solution of eqns. 1 and 2 and their applications were described in detail in Part I[1].

EXPERIMENTAL

Gel permeation chromatography

All GPC measurements were carried out with the same apparatus as and under experimental conditions similar to those in Part I[1]. Experimental data[1] obtained on a column packed with CPG-10-1000 porous glass were also used in calculations in this work. For this reason, only some essential and novel experimental data are reported in this paper.

A column of the same dimensions as in Part I (CPG-10-1000) was packed with Porasil E porous silica gel (Waters Assoc., Milford, Mass., U.S.A.) of particle size 40–70 μm. The flow-rate of tetrahydrofuran (THF) was 0.341 ml/min. Elution volumes were measured using a 1.704-ml siphon; the reproducibility of elution volumes of the maximum of the elution curves was the same as in Part I, *i.e.*, ±0.1 count. The solutions were injected from the same injecting system using a calibrated 0.67-ml loop.

Polystyrene samples

Polystyrene (PS) standards (Waters Assoc.) with a narrow molecular weight distribution and several high-molecular-weight PS samples prepared by anionic polymerization were used in the preliminary determination of the calibration graph for the column packed with Porasil E. The calibration graph is shown in Fig. 1. In further work, only selected PS standards, denoted similarly as in Part I[1] as PS

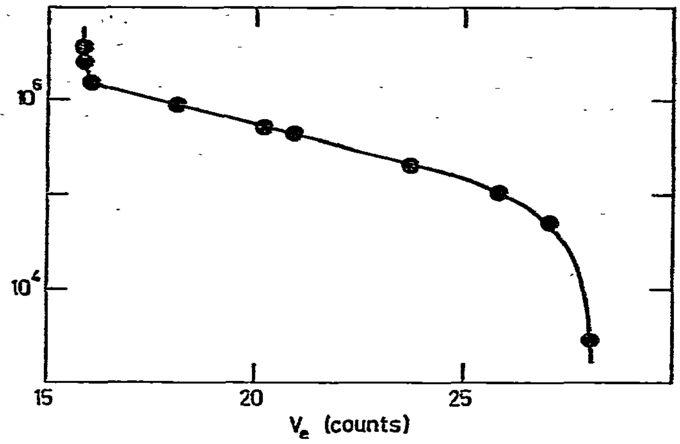

Fig. 1. Calibration graph for the separation column (Porasil E) at a concentration $g_0 = 0.05\%$ (w/v).

1, PS 3, PS 4 and PS 6, were investigated. Their weight-average molecular weights, $\bar{M}_w$, were 2,610,000 (PS 1), 867,000 (PS 2), 498,000 (PS 3) and 200,000 (PS 6). Calculations also required a knowledge of the initial conditions after injection of the sample, *i.e.*, concentration (g_I) and dispersion (σ_I) at the beginning of the column, the former not being identical with the original concentration in the injection loop (g_0) and σ_0 being equal to one quarter of the injected volume. As the injection system remained unchanged, the same values calculated for the injection of the whole loop (0.67 ml) as in Part I were used in the calculations.

RESULTS AND DISCUSSION

Eqn. 2 shows that the elution volume of the polymer under investigation increases by a contribution due to the viscosity effect, regardless of molecular weight, This assumption was proved to a good approximation in Part II[2]. Quantitatively. this contribution can be evaluated by using data on the concentration dependence of the elution volume of totally excluded polymeric samples —standard PS 1 for both columns in our work (*cf.*, Fig. 2 in Part I[1] and Fig. 1 in this paper). The contribution to overall changes in the elution volumes accompanying changes in concentration caused by the expansion of macromolecular coils of the PS standards, the macromolecules of which may penetrate pores of the column packing, was calculated by using a scheme given in Part I[1], with eqn. 1 as the starting expression.

Unlike in Part I[1], average elution volumes (V_{av}) and dispersions (square roots from variance) defined by[2]

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

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

were used in the calculations, instead of the elution volume of the maximum of the elution curve, V_e, and the graphically determined total dispersion of the elution curve at the end of the column, σ_T. In the equations used, h_i are heights of the chromatogram from the baseline in the respective elution volumes V_i. Experience gained in the evaluation of the elution curves measured under extreme experimental conditions in Part II has indicated[2] that such a method of evaluation is justified, and for deformed elution curves that exhibit several sharp maxima it is the only possible method that leads to correct results.

The procedure just mentioned was used to re-calculate experimental data from Part I[1], obtained on a column packed with CPG-10-1000 porous glass. The results are summarized in Table I. The ΔV values given in Table I are the differences between the average elution volume and the elution volume of the maximum of the elution curve. It can be seen that while ΔV values of the standards PS 3 and PS 4 lie on the average, within the limits of experimental error, for the standard PS 1 ΔV increases with increasing concentration of the injected sample. These differences are due to the increasing tailing of the resulting elution curve of the totally excluded standard PS 1. The resulting elution curves of the standards PS 3 and PS 4 (and also PS 6) were symmetrical to a good approximation (judging by the values of the third moments around the mean of the elution curves μ_3).

Important differences have also been observed between the $\Delta\sigma$ values of the standard

TABLE I

EXPERIMENTAL AND CALCULATED DATA OF THE DEPENDENCE OF THE ELUTION VOLUME ON CONCENTRATION FOR A COLUMN PACKED WITH CPG-10-1000 POROUS GLASS

Sample	g_0 (%)	*Experimental values**				*Calculated values**			
		V_{av}	ΔV	σ_T	$\Delta\sigma$	V_v	ΔV_v	V_{eo}**	V_e***
PS 1	0.8	20.8	+2.6	3.45	+0.75	3.4	+2.75		
	0.4	19.3	+1.3	2.53	+0.70	1.9	+1.45		
	0.2	18.7	+0.8	2.12	+0.49	1.3	+0.95		
	0.1	18.3	+0.5	1.68	+0.18	0.9	+0.65		
	0.05	17.8	+0.1	1.48	−0.02	0.4	+0.25		
	0.025	17.5	−0.1	1.35	−0.03	0.1	+0.05		
	0							17.4	
PS 3	0.4	22.9	+0.1	2.01	+0.18	1.10	+0.92		21.49
	0.2	22.1	−0.2	1.69	−0.01	0.62	+0.53		21.38
	0.1	21.8	0	1.54	−0.09	0.33	+0.28		21.31
	0.05	21.6	−0.1	1.50	−0.13	0.17	+0.15		21.27
	0.025	21.5	−0.1	1.48	−0.15	0.08	+0.07		21.24
	0							21.4	
PS 4	0.8	25.7	−0.1	2.34	+0.26	1.30	+1.08		24.40
	0.4	25.2	0	1.95	0	0.74	+0.63		24.27
	0.2	24.6	−0.1	1.88	+0.03	0.38	+0.32		24.18
	0.1	24.3	−0.1	1.74	−0.11	0.20	+0.17		24.14
	0.05	24.0	−0.2	1.56	−0.14	0.11	+0.09		24.11
	0.025	23.8	−0.3	1.56	−0.14	0.05	+0.04		24.10
	0							23.8	
PS 6	0							28.9	

* V counts.

** Extrapolated values at $g = 0$.

*** Calculated from eqn. 15 in Part I[1].

PS 1 and the other PS standards. The $\Delta\sigma$ values are the difference between the σ_T values calculated by means of eqn. 4 and those which were previously determined graphically[1]. These differences eventually led to a quantitative underestimation of V_v due to the viscosity effect, as can be seen from the ΔV_v values, which are the difference between the V_v values calculated here and those originally calculated[1].

The elution volumes (V_{e0}) extrapolated to the concentration $g_I = 0$ were calculated by a procedure described in Part I[1]. The constants P and Q at $g_I = 0$ for eqn. 1 and the elution volumes V_e according to eqn. 15 in Part I[1] were also calculated. The fact that some V_e values in Tables I and II are lower than V_{e0} for the same standard follows from the linear regression of experimental values subjected to experimental errors.

The difference between V_v, V_e and V_{av} values at extreme concentrations of the standards PS 3 and PS 4 were used for determining the contribution of the viscosity effect and of the effect of the expansion of macromolecular coils to the total concentration effects. The differences between V_e values calculated here and in Part I are virtually the same (±10% relative). The ratio of the contribution of the viscosity effect to that due to the expansion of macromolecular coils to separation is approximately 4:1. At the same time, the contribution of the sum of two calculated contributions to the total concentration effect (*i.e.*, to the change in the elution volume caused by a change in the concentration of the injected sample) is *ca.* 87%.

Experimental data measured on a column packed with Porasil E silica gel were treated by employing the method described in this work and by a procedure described in Part I[1]. Table II summarizes results obtained with Porasil E. In this instance also there are differences between ΔV, $\Delta\sigma$ and ΔV_v for the standard PS 1 and for the other PS standards, but they are less than those with a column packed with CPG-10-1000 porous glass. Small tailings were observed with all PS standards at all concentrations (with the exception of the highest concentrations of PS 1). On the other hand, however, lower σ_T values were attained compared with the preceding column. The ratio of the contribution of the viscosity effect to that due to the effect of the expansion of macromolecules to separation is again approximately 4:1 to 3:1. The contribution of the two calculated contributions to the total concentration effect is about 100%.

By comparing results in Tables I and II, it can be seen that with an asymmetrical (skewed) elution curve there is a distinct underestimation of the viscosity effect if experimental data are treated in a simple way, *i.e.*, using the elution volumes of the maximum of the elution curves and graphically determined σ_T values. The efficiencies of the columns used, expressed as the number of theoretical plates per column, $N = (V_{av}/\sigma_T)^2$, and calculated from the elution curves of standards PS 1, PS 3 and PS 4 at all concentrations used, are given in Table III. In almost all instances N is smaller for a column packed with porous glass. The lower efficiency of the column is obviously related to the particularly distinct differences between the results when different methods of evaluation of the concentration effects are used.

The results indicate that the viscosity effect in the interstitial volume plays a decisive role in the overall concentration effect under given experimental conditions. Its contribution is approximately four times larger than that due to the effect of the concentration expansion of macromolecules. On average, the sum of both contributions is very close to 100%, which can be regarded as a very good result because

TABLE II

EXPERIMENTAL AND CALCULATED DATA OF THE DEPENDENCE OF THE ELUTION VOLUME ON CONCENTRATION FOR A COLUMN PACKED WITH PORASIL E SILICA GEL

Sample	g_0 (%)	*Experimental values**				*Calculated values**			
		V_{ex}	ΔV	σ_T	$\Delta\sigma$	V_v	ΔV_v	V_{eo}**	V_e***
PS 1	0.8	19.1	+1.1	3.11	+0.32	3.6	+0.6		
	0.4	17.4	+1.2	1.86	+0.20	1.9	+0.7		
	0.2	16.6	+0.6	1.50	+0.15	1.1	+0.1		
	0.1	16.4	+0.8	1.50	+0.34	0.9	+0.3		
	0.05	16.0	+0.5	1.20	+0.38	0.5	0		
	0.025	15.8	+0.5	0.92	+0.13	0.3	0		
	0							15.5	
PS 3	0.4	19.3	+0.5	1.63	+0.10	1.13	+0.23		18.12
	0.2	18.5	+0.2	1.19	−0.09	0.72	+0.21		18.03
	0.1	18.4	+0.3	1.18	+0.02	0.35	+0.08		17.95
	0.05	18.2	+0.4	1.08	−0.02	0.19	+0.05		17.91
	0.025	18.1	+0.3	1.19	+0.10	0.09	+0.02		17.89
	0							18.0	
PS 4	0.8	22.0	+0.4	1.71	−0.29	1.48	+0.52		20.44
	0.4	20.8	+0.2	1.36	−0.06	0.87	+0.24		20.32
	0.2	20.3	+0.3	1.22	−0.04	0.47	+0.13		20.23
	0.1	20.3	+0.5	1.12	−0.10	0.25	+0.08		20.18
	0.05	20.2	+0.2	1.11	−0.10	0.12	+0.03		20.15
	0							19.9	
PS 6	0							23.9	

* *V* counts.
** Extrapolated values at $g = 0$.
*** Calculated from eqn. 15 in Part I[1].

TABLE III

EFFICIENCY OF COLUMNS AS A FUNCTION OF CONCENTRATION OF VARIOUS INJECTED POLYSTYRENE STANDARDS

Sample	g_c (%)	*No. of theoretical plates per column (N)*	
		CPG-10-1000	*Porasil E*
PS 1	0.8	36	38
	0.4	58	88
	0.2	78	123
	0.1	118	120
	0.05	145	178
	0.025	168	295
PS 3	0.4	129	126
	0.2	171	242
	0.1	200	243
	0.05	207	284
	0.025	211	231
PS 4	0.8	121	166
	0.4	167	234
	0.2	171	277
	0.1	195	329
	0.05	237	331

some simplifications were implicitly introduced into the calculations. The calculations were based on concentration changes along the column in the maximum of the zone, which is regarded as Gaussian. Although the zone loses its Gaussian character at higher concentrations or viscosities, there is a linear relationship between the mean elution volume and specific viscosity[2]. Departures from linearity appear only at extreme concentrations and higher flow-rates. A Gaussian shape of the zone is always assumed in the calculations for the sake of simplicity. Such simplification should affect the calculation of both contributions to the same extent, so that their ratio could be regarded, to a good approximation, as correct.

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REFERENCES

1 J. Janča, *J. Chromatogr.*, 134 (1977) 263.
2 J. Janča and S. Pokorný, *J. Chromatogr.*, 148 (1978) 31.