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CONCENTRATION EFFECTS IN GEL PERMEATION CHROMATOGRAPHY

I. POLYMER SOLUTION PROPERTIES

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SUMMARY

Equations have been derived that give a quantitative description of the processes that occur in a chromatographic column, leading to a concentration dependence of the elution volume in the gel permeation chromatography of polymers. The concentration dependence was studied experimentally on rigid porous glass, that is, under conditions when the size of the accessible pores is not influenced by a change in the thermodynamic properties of the solvent. The experimental data were correlated with the relationships obtained in the investigation.

INTRODUCTION

The dependences of the elution volume (V_e) and the width of the elution curve (w) on the concentration (g) and amount of polymer solution injected in gel permeation chromatography (GPC) have been observed by many workers. According to Waters[1], the increase in V_e with increasing concentration was caused by the higher viscosity (η) of injected solution. Boni and co-workers[2,3] observed that the change (dV_e/dg) was a linear function of the logarithm of molecular weight (log M). For the correlation with log [η], they obtained a single linear dependence for various polymers. By plotting V_e as a function of the weight of polymer injected, they succeeded (unlike Waters[1]) in drawing a single straight line through the experimental points for various concentrations and injection times. These workers put forward a hypothesis that the viscosity effect appeared predominantly in the interstitial volume. Similar experimental results were obtained by Lambert[4], except that the dependence of V_e on the weight of polymer injected was not linear and the curvature became greater with increasing molecular weight.

The hypothesis of the viscosity effect was supported by Goetze *et al.*[5], who injected the polymer sample in a solvent that had a higher relative viscosity than that used in GPC. They inferred that the viscosity effect caused changes in V_e, but the overall phenomenon could not be assigned merely to this. Moore[6] explained the

viscosity effect as "viscous fingering". Rudin[7] pointed out that the effective hydrodynamic volume of the macromolecule decreases with increasing concentration. A hydrodynamic volume, proportional to the product $[\eta] \cdot M$, is used as a universal calibration parameter in the GPC of polymers[8].

The effect of concentration must be considered when constructing a universal calibration graph[9]. This hypothesis on the effect of concentration on V_e was supported by other workers[10,11], who regarded the effect of concentration on V_e in the Θ solvent used in the GPC separation as being very small. The mutual arrangement of the individual columns also seems to influence the concentration dependence of V_e (ref. 12). The increase in w with increasing concentration and volume of sample injected was observed by several workers[13–15]. Recently, Baghurst *et al.*[16] published an interesting theory on the effect of concentration on V_e with a swelling gel used as the column packing. They assumed that osmotic shrinkage of the gel packing occurred in the eluting zone.

The above survey indicates that some confusion and opposing views still exist on the effects of concentration and amount of polymer solution injected on GPC results.

THEORETICAL

The factor that determines V_e is the effective hydrodynamic volume of the macromolecular coil, which is a function of the concentration, g. Consequently, a universal calibration parameter, $v\varepsilon_0$, holds at $g = 0$, and $v\varepsilon$ holds at $g > 0$, where v is the volume of unswollen macromolecule and ε_0 and ε are the effective volume factors of swelling at a concentration extrapolated to zero and at a given real concentration, respectively. In the central part of the calibration graph it usually holds that

$$V_e = P + Q \ln (v\varepsilon) \tag{1}$$

where P and Q are constants. Also, for a given polymer[7]

$$\frac{1}{\varepsilon} = \frac{1}{\varepsilon_0} + \frac{g}{0.507\varrho} \cdot \frac{\varepsilon_0 - \varepsilon_x}{\varepsilon_0} \tag{2}$$

$$\varepsilon_0 = \frac{KM^a\varrho}{2.5} \tag{3}$$

$$\varepsilon_x = 2.60 + (0.34 \cdot 10^{-3}) \cdot \frac{M}{M_0} \tag{4}$$

and

$$v = \frac{M}{\varrho N_0} \tag{5}$$

where ϱ is the density of an amorphous polymer at a given temperature, ε_x is the critical volume factor, K and a are the constant and the exponent of the Mark–Houwink equation, respectively, M_0 is the molecular weight of the repeating monomer unit and

N_0 is Avogadro's number. The elution curve at an arbitrary position in the column can be described by a Gaussian function[17]:

$$F(x) = \frac{1}{\sigma\sqrt{2\pi}} \cdot e^{-\frac{1}{2}\left[\frac{(x-u)}{\sigma^2}\right]^2} \tag{6}$$

where σ is the standard deviation of the elution curve and u is the coordinate of the peak of the elution curve. We assume for the sake of simplicity that the moment of injection can also be described by the function $F(x)$. According to Hendrickson[18], for a homogeneously packed column

$$\sigma_T^2 = \sigma_I^2 + L\sigma_U^2 \tag{7}$$

where σ_T characterizes the overall width of the elution curve at the column end, σ_I is the width of injection, L is the column length and σ_U is the contribution of the unit length of the column to dispersion. If σ_u is the width of the elution curve in the coordinate u of the column, then

$$\sigma_u^2 = \sigma_I^2 + u \cdot \frac{\sigma_T^2 - \sigma_I^2}{L} \tag{8}$$

Let us note the change in the concentration of eluted monodisperse polymer along the column at the peak of the elution curve. For $F(x)$ at this point we have

$$F(x)_{max} = \frac{1}{\sigma\sqrt{2\pi}} \tag{9}$$

For the concentration at the peak of the elution curve and for the coordinate u of the column

$$g = k \cdot \frac{1}{\sigma_u\sqrt{2\pi}} \tag{10}$$

The constant k can be determined from the starting conditions at the moment of injection, when the concentration of the injected sample, g_I, is known.

Assuming an instantaneous change in the effective hydrodynamic volume with a change in the concentration, the elution volume can be calculated by using the equation

$$V_e = P + Q \ln v + \frac{Q}{L}\int_0^L \ln \varepsilon \, du \tag{11}$$

If we define the constants

$$A = \frac{1}{\varepsilon_0} \tag{12}$$

and

$$B = \frac{1}{0.507 \cdot \varrho} \cdot \frac{\varepsilon_0 - \varepsilon_x}{\varepsilon_0} \tag{13}$$

then, by substituting into eqn. 11 from eqns. 2, 8, 10, 12 and 13, we obtain

$$V_e = P + Q \ln v - \frac{Q}{L} \int_0^L \ln \left[A + \frac{B g_I \sigma_I}{\sqrt{\sigma_I^2 + u \cdot \frac{\sigma_T^2 - \sigma_I^2}{L}}} \right] \mathrm{d}u \tag{14}$$

By solving eqn. 14:

$$V_e = P + Q \left[\ln v + \frac{B^2 g_I^2 \sigma_I^2}{(\sigma_T^2 - \sigma_I^2) A^2} \cdot \ln \left(\frac{\sigma_T A + B g_I \sigma_I}{\sigma_I A + B g_I \sigma_I} \right) - \frac{B g_I \sigma_I}{(\sigma_T + \sigma_I) A} + \right.$$

$$\left. + \frac{\sigma_I^2}{\sigma_T^2 - \sigma_I^2} \cdot \ln (A + B g_I) - \frac{\sigma_T^2}{\sigma_T^2 - \sigma_I^2} \cdot \ln \left(\frac{\sigma_T A + B g_I \sigma_i}{\sigma_T} \right) \right] \tag{15}$$

A 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:

$$V_v = \frac{k'}{L} \int_0^L \eta_{\mathrm{spec}} \, \mathrm{d}u \tag{16}$$

where k' is the proportionality constant. By substituting from the Huggins equation for η_{spec} in eqn. 16 and using eqns. 8 and 10, we obtain

$$V_v = \frac{k'[\eta] g_I \sigma_I}{L} \cdot \int_0^L \frac{\mathrm{d}u}{\sqrt{\sigma_I^2 + u \cdot \frac{\sigma_T^2 - \sigma_I^2}{L}}} +$$

$$+ \frac{k' k_H [\eta]^2 g_I^2 \sigma_I^2}{L} \cdot \int_0^L \frac{\mathrm{d}u}{\sigma_I^2 + u \cdot \frac{\sigma_T^2 - \sigma_I^2}{L}} \tag{17}$$

where k_H is the Huggins constant for the respective polymer–solvent system and $[\eta]$ is intrinsic viscosity.

The solution of eqn. 17 gives

$$V_v = k' \cdot \left(\frac{2[\eta] g_I \sigma_I}{\sigma_T + \sigma_I} + \frac{2 k_H [\eta]^2 g_I^2 \sigma_I^2}{\sigma_T^2 - \sigma_I^2} \cdot \ln \frac{\sigma_T}{\sigma_I} \right) \tag{18}$$

The constant k' can be calculated by using eqn. 18 using the elution volumes at different concentrations of excluded macromolecules.

EXPERIMENTAL

Gel permeation chromatography

All GPC measurements were carried out with an apparatus built at the Institute of Macromolecular Chemistry of the Czechoslovak Academy of Sciences in Prague and provided with an R-403 refractometer (Waters Assoc., Milford, Mass., U.S.A.). The separation system was maintained thermostatically at 25 $\pm$ 0.1°. Tetrahydrofuran (THF) distilled from copper(I) chloride and potassium hydroxide under an atmosphere of nitrogen was used as solvent. The flow-rate of THF was 0.375 ml/min. The elution volumes were measured by using a calibrated siphon of volume 1.704 ml. The reproducibility of V_e with one separation column was better than $\pm$0.1 count under the conditions used. The solutions were injected from a calibrated loop of volume 0.67 ml. The standard column was 120 cm long, with an I.D. of 0.8 cm. Controlled Pore Glass (Electro-Nucleonics, Fairfield, N.J., U.S.A.), Type GPC-10-1000, grain size 200–400 mesh, was used as the column packing.

Polystyrene samples

Polystyrene (PS) standards (Waters Assoc.), with a very narrow distribution were used. The designations of the PS standards and their molecular parameters are given in Table I. The Huggins constants, k_H, for the PS standards were calculated from our earlier experimental data[19]. The final k_H values are listed in Table I. The

TABLE I

MOLECULAR PARAMETERS OF POLYSTYRENE STANDARDS AND HIGH-MOLECULAR-WEIGHT FRACTIONS OF ANIONIC POLYSTYRENE

Type of sample	*Designation of sample*	$\bar{M}_w \cdot 10^{-3}$	k_H	$v \cdot 10^{19}$	ε_0	ε_x	$A \cdot 10^3$	B
		Producer's data						
Polystyrene standards	PS 1	2610	—	41.66	194.1	11.038	5.152	1.789
	PS 2	2145	0.352	—	—	—	—	—
	PS 3	867	0.365	13.84	88.1	5.403	11.35	1.780
	PS 4	498	—	7.949	59.18	4.210	16.90	1.762
	PS 5	411	0.367	—	—	—	—	—
	PS 6	200	—	3.192	30.77	3.247	32.50	1.696
	PS 7	173	0.364	—	—	—	—	—
	PS 8	98.2	0.428	1.567	18.48	2.917	54.12	1.597
		Experimental values						
Polystyrene fractions	F 1	3700						
	F 2	3000						
	F 3	1800						
	F 4	1300						
	F 5	1120						

subsequent calculations were carried out using $k_H = 0.362$, which is an arithmetic mean from the k_H values in Table I for four high-molecular-weight PS standards. The exclusion limit of the column was also determined by using a few high-molecular-weight PS samples with a narrow distribution ($\bar{M}_w/\bar{M}_n \approx 1.2$) prepared by the anionic polymerization. The weight-average molecular weights ($\bar{M}_w$) of these samples were determined by light scattering and are given in Table I.

All calculations were carried out by using the Mark–Houwink equation[19]:

$$[\eta] = 1.17 \cdot 10^{-2} \cdot M^{0.717} \tag{19}$$

which holds for linear PS in THF at 25°. The density of amorphous PS, $\varrho_{23°} = 1.04$ g/cm^3, was taken from the literature[20].

RESULTS AND DISCUSSION

The injection loop and the separation column were connected with a capillary 140 cm long. As dispersion occurs, g_I and σ_I at the beginning of the separation column need not have been identical with the original concentration in the injection loop, g_0, and with σ_0 equal to the injected volume. When the injection loop was connected directly with the differenitial refractometer, the total length of capillary from the orifice of the injection valve to the measuring cell of the refractometer was 70 cm (an essential part of which is the length of the heat exchanger of the refractometer). If the solutions of various PS standards at concentrations from 0.05 to 0.4% (w/v) were injected into the system without a separation column and connecting capillary and into the system with a connecting capillary, the molecular weight and concentration were found to have no influence on the dispersion.

Experimental data are summarized in Table II. Both the total volume of the loop and the partial volume were injected; in the latter instance the injection times were 60 and 30 sec. The chromatograms exhibited sharp maxima in all instances, as illustrated in Fig. 1a; it could not be decided, therefore, if the maximum response of the refractometer corresponded to the concentration g_0. If, however, the original loop

TABLE II

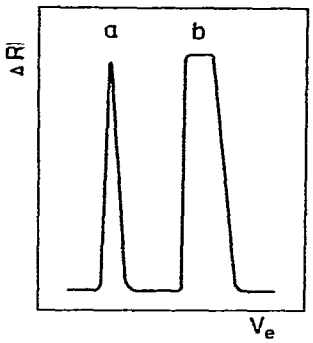
DETERMINATION OF DISPERSION OF INJECTION SYSTEM

Injection system	*Volume injected (ml)*	h^*	$\sigma_T'^{**}$	σ_0^{***}	$V_{e\ max}$ *(counts)*
Without connecting capillary	0.670	44	0.188	0.0983	0.50
	0.375	43	0.113	0.0550	0.40
	0.188	32	0.083	0.0275	0.33
	1.696	48	—	—	—
With connecting capillary	0.670	29	0.300	0.0983	1.00
	0.375	19	0.250	0.0550	0.90
	0.188	9.5	0.238	0.0275	0.85

* Height of chromatogram (arbitrary units).

** Width of chromatogram (counts).

*** Width of unspread injection (counts), calculated from injected volume and siphon volume.



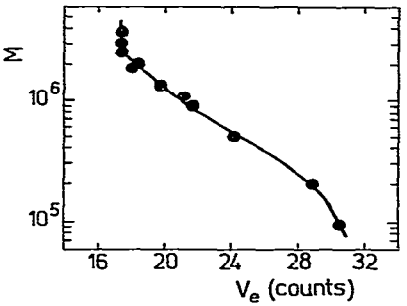


Fig. 1. Elution curve obtained with injections of (a) 0.67 ml and (b) 1.696 ml of polymer solutions in the same concentration from the injection system into the refractometer cell without a connecting capillary.

Fig. 2. Calibration graph for the separation column (CPG-10-1000) at a concentration $g_0 = 0.05\%$ (w/v).

of volume 0.67 ml was replaced only for this purpose with a loop of volume 1.696 ml, a plateau (Fig. 1b) appeared in the maximum of the chromatogram after injecting the whole volume into the system without a connecting capillary. The height of the chromatogram between the baseline and the plateau, h_0, undoubtedly corresponds to the concentration g_0. The maximum concentration with an injection of 0.67 ml in the given instance was 92% of the concentration g_0.

Eqn. 7 was assumed to be valid also for the capillary injection system. In this way, σ_I values were calculated for all injection times by interpolation from the experimental data in Table II. When less than the whole volume of the loop was injected, one had to bear in mind that spreading proceeded asymmetrically only from the front of the injected volume during the whole injection time.

The σ_I values calculated as indicated above were 0.25 count for an injection of 0.67 ml, 0.20 count for an injection of 0.375 ml and 0.81 count for an injection of 0.188 ml. The g_I values were calculated from the equation

$$g_I = g_0 \cdot \frac{\sigma'_T}{\sigma_I} \cdot \frac{h'}{h_0} \tag{20}$$

where σ'_T and h' are the width and height of the chromatogram in the case of injection into the system without a separation column and connecting capillary.

Viscosity effect

The calibration graph determined by injecting 0.67 ml of solutions of the PS 1–PS 8 standards and of the F 1–F 5 fractions in concentrations of 0.05% (w/v) (Fig. 2) showed that the molecular weight of the standard PS 1 already lay above the exclusion limit of the packing used. All experimental data given in Table III were obtained by injecting 0.67 ml of solutions of the PS standards with concentrations g_0 ranging from 0.8 to 0.025% (w/v). The constant $k' = 1.176$ was calculated from experimental data for the standard PS 1 having g_0 concentrations of 0.8 and 0.025% (w/v). The V_D values calculated for the other PS standards are also summarized in Table III.

TABLE III

EXPERIMENTAL AND CALCULATED DATA ON THE DEPENDENCE OF ELUTION VOLUME ON CONCENTRATION

Sample	g_0 (%)	*Experimental values*		*Calculated values*		
		V_e *(counts)*	σ_T *(counts)*	V_v *(counts)*	V_{e0}* *(counts)*	V_e** *(counts)*
PS 1	0.8	18.2	2.70	0.65		
	0.4	18.0	1.83	0.45		
	0.2	17.9	1.63	0.35		
	0.1	17.8	1.50	0.25		
	0.05	17.7	1.50	0.15		
	0.025	17.6	1.38	0.05		
	0				17.55	
PS 3	0.4	22.8	1.83	0.18		21.70
	0.2	22.3	1.70	0.09		21.57
	0.1	21.8	1.63	0.05		21.50
	0.05	21.7	1.63	0.02		21.45
	0.025	21.6	1.63	0.01		21.43
	0				21.50	
PS 4	0.8	25.8	2.08	0.22		24.54
	0.4	25.2	1.95	0.11		24.39
	0.2	24.7	1.85	0.06		24.30
	0.1	24.4	1.85	0.03		24.25
	0.05	24.2	1.70	0.02		24.23
	0.025	24.1	1.70	0.01		24.22
	0				24.10	
PS 6	0.4	29.2	1.93	0.06		28.88
	0.2	28.9	1.88	0.03		28.84
	0.1	28.9	2.08	0.01		28.81
	0.05	28.9	2.25	0.01		28.80
	0.025	28.9	2.38	0.00		28.79
	0				28.85	

* Extrapolated values at $g = 0$ (counts).
** Calculated from eqn. 15 (counts).

The results show that the viscosity effect in the interstitial volume represents only *ca.* 12–15% of the total concentration effect under the given experimental conditions.

Effect of the expansion of macromolecular coils

The constants P and Q and the elution volumes V_{e0} extrapolated to the concentration $g = 0$ were calculated by using the linear regression method from the experimental V_e values (on subtracting the V_v values) of the standards PS 3, 4 and 6 (lying on the linear part of the calibration graph) at various concentrations and from the respective expressions given in parentheses on the right-hand side of eqn. 15 (Table III). By using the V_{e0} and $v\varepsilon_0$ values of the standards PS 3, 4 and 6 and employing the linear regression method, we calculated the constants $P = -85.89$ and $Q = -2.928$ in eqn. 1 which ensues from eqn. 15 at $g_I = 0$. These constants P and

Q were not identical with those calculated from the concentration dependence of V_e of the individual PS standards. The constants P and Q valid for the linear part of the calibration graph extrapolated to zero concentration were used in calculating V_e from eqn. 15, for the standards PS 3, 4 and 6 at the given concentrations. The results summarized in Table III show that the change in the effective dimensions of the macromolecular coil due to a change in concentration participates in the change in V_e by about 20–30%.

The disagreement between the experimental and calculated data cannot be attributed to experimental error. Even in the least favourable case the results of calculations are not affected in a decisive manner, as has been verified by intentional alterations of the input data. One of the possible explanations is that the change in the effective hydrodynamic volume with a change in concentration is not instantaneous. However, we found that the elution volumes of the standard PS 4 dissolved in the Θ solvent and injected within a concentration range from 0.8–0.1% (w/v) were completely identical with the V_e value of the same standard dissolved in THF. Although macromolecules are separated from the Θ solvent during elution, an approximately 2.5-fold difference between the effective hydrodynamic volumes of the PS standard in THF and the Θ solvent at the beginning of separation should have been reflected in a change in V_e. A THF–methanol mixture (71.3:28.7, v/v) was used as the Θ solvent at 25° (ref. 21).

Effect of injected volume

The calculation of V_e for various injected volumes starting with half of the injected volume[2,3] is justified, as it follows from the theory of chromatography that the effective column length is shortened by half of the width of the injected volume[22]. The elution volumes of the standard PS 4 at concentrations of 0.4–0.05% (w/v) for two different injected volumes are given in Table IV. The differences between V_e given here and V_e for injection of the whole loop volume (Table III) correspond within the limits of experimental error to the difference between half the values of the injected volumes for an injection of 0.375 ml. For an injection of 0.188 ml the differences between V_e are higher than corresponds to the difference between half the values of the injected volumes. However, the g_I values are lower than in the preceding instances, as demonstrated by Table II. Indeed, the V_e values in Table IV for an injection of 0.188 ml can be adequately correlated, within the limits of experimental error, with the V_e values in Table III for half the values of the concentrations g_0.

TABLE IV

EFFECT OF AMOUNT OF SAMPLE INJECTED ON ELUTION VOLUME

Volume injected (ml)	*g. (%)*	*V_e (counts)*	*σ_T (counts)*
0.375	0.4	24.9	1.90
	0.2	24.5	1.85
	0.1	24.3	1.80
	0.05	24.2	1.70
0.188	0.4	24.5	1.90
	0.2	24.2	1.85
	0.1	24.0	1.80

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