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CONCENTRATION EFFECTS IN GEL PERMEATION CHROMATOGRAPHY

IV. CONTRIBUTION OF VISCOSITY PHENOMENA AND MACROMOLECULAR EXPANSION

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SUMMARY

The changes in elution volumes with the changes in the concentration of an injected polymer solution are caused by several contributing effects. Under model conditions, it is possible to assume only the effect of a viscosity gradient in a zone moving along the column and the effect of the concentration dependence of the hydrodynamic volume of a macromolecular coil. The non-Gaussian shape of the zone and the dependence of the width of the elution curve on concentration are factors that complicate the theoretical treatment of concentration effects. The described physico-chemical model allows to evaluate the ratio of mentioned two contributions in the concentration dependence of elution volumes. According to this model, the contribution of the concentration dependence of the hydrodynamic volume does not exceed about 20% of the total change in elution volume with the varying concentration under real experimental conditions. The efficiency of the columns used and the total injected volume of the polymer solution affect this ratio only negligibly. It is obvious from a comparison with earlier results that these conclusions are not fundamentally changed even by the revised model of the concentration dependence of the swelling factor.

INTRODUCTION

In previous papers in this series¹⁻³, we considered some aspects of concentration effects in the gel permeation chromatography (GPC) of polymers. Some relationships were derived in Part I¹ that permit the quantitative description of these effects under real experimental conditions, *i.e.*, the dependence of the elution volume on the concentration of an injected solution of a polymer sample. The concentration effects were investigated both theoretically and experimentally under model conditions, when only the effect of the viscosity of the polymer solution moving along a column and the effect of the concentration-dependent expansion of macromolecular coils in solution were substantially operative. The quantitative description of the

latter effect originated from the theoretical model of the dependence of the effective hydrodynamic volume on polymer concentration published by Rudin⁴.

Rudin and Wagner⁵ have recently revised the earlier work⁴ and developed a new theoretical model which is less empirical and especially takes into account the behaviour of polymers in a thermodynamically poor solvent (theta solvent). This paper deals with the consequences for our theoretical model¹ that follow from the revised concepts of Rudin and Wagner⁵. Further, we deal with the problem of the ratio of individual contributions to the overall concentration effect, as follows from the proposed model and with correlations of some new experimental results.

THEORETICAL

The hydrodynamic volume of a solvated polymer is defined by a product $v\varepsilon$, where v is the volume of the unswollen coil and ε is the dimensionless swelling factor. The dependence of the swelling factor on concentration, g , is given by the relationship⁵

$$\frac{1}{\varepsilon} = \frac{1}{\varepsilon_0} + \frac{g}{g_x} \left(\frac{\varepsilon_0 - 1}{\varepsilon_0} \right) \quad (1)$$

where the swelling factor, at infinite dilution ($g = 0$), $\varepsilon = \varepsilon_0$, is⁵

$$\varepsilon_0 = \frac{[\eta]}{[\eta]_\theta} = \frac{KM^{a-0.5}M_0^{3/2}}{0.25\beta^3} \quad (2)$$

and $\varepsilon = 1$ at $g = g_x$ when

$$[\eta]_\theta = K_\theta M^{0.5} \quad (3)$$

and

$$K_\theta = 2.5 \cdot 10^{-1} \cdot \frac{\beta^3}{M_0^{3/2}} \quad (4)$$

where the intrinsic viscosity, $[\eta]$, and the molecular weight, M , are determined, under the given conditions, by the Mark-Houwink equation with a constant K and an exponent a :

$$[\eta] = KM^a \quad (5)$$

which under theta conditions is reduced to eqn. 3. M_0 in eqns. 2 and 4 represents half of the molecular weight of the repeating vinyl monomer unit and β (Å) is the effective length of the bond ($\beta = 4.96$ Å for polystyrene⁶).

The concentration g_x corresponds to the critical volume fraction, Φ_x , of the polymer in solution, at which the dimensions of the solvated macromolecules are identical with the dimensions under theta conditions. Also

$$g_x = \frac{\Phi_x M}{N_0 v} \quad (6)$$

and

$$v = \frac{4\pi[\eta]_0 M}{3\Phi'} \quad (7)$$

where N_0 is Avogadro's number and the Flory constant⁷ $\Phi' = 3.1 \cdot 10^{24}$.

We assumed that the axial concentration profile, *i.e.*, the distribution of the polymer concentration in individual volume elements along the column axis, can be described by a Gaussian function:

$$F(x) = \frac{1}{\sigma \sqrt{2\pi}} \cdot e^{-\frac{1}{2} \left[\frac{(x-u)^2}{\sigma^2} \right]} \quad (8)$$

where σ is the standard deviation of the Gaussian function and u is the coordinate of the position of the maximum of the concentration profile in the direction of the column axis. For the sake of simplicity of mathematical formulation, we used the dependence of the concentration at the maximum of the Gaussian concentration profile on the position of the maximum for the zone moving along the column, which is characterized by

$$g = k \cdot \frac{1}{\sigma_u \sqrt{2\pi}} \quad (9)$$

Now, for the standard deviation of the concentration profile, σ_u , at the position of the maximum with coordinate u , we have

$$\sigma_u^2 = \sigma_I^2 + u \cdot \frac{\sigma_T^2 - \sigma_I^2}{L} \quad (10)$$

where σ_I and σ_T characterize the standard deviations of the injected portion (which for simplicity is also approximated by a Gaussian function) and of the elution curve at the end of a column of length L (if the end-effect is neglected⁸), respectively. The empirical constant k can be determined from the known initial conditions g_I and σ_I at the moment of injection of the sample solution on the beginning of the column:

$$k = g_I \sigma_I \sqrt{2\pi} \quad (11)$$

This means that the concentration g is given by a simple expression:

$$g = g_I \cdot \frac{\sigma_I}{\sigma_u} \quad (12)$$

The elution volume of a polymer, V_e , can be formulated as follows:

$$V_e = P + \frac{Q}{L} \int_0^L \ln(v\varepsilon) du + \frac{k'}{L} \int_0^L \eta_{spcc} du \quad (13)$$

The first two terms on the right-hand side of eqn. 13 express mainly the relationship between the logarithm of the hydrodynamic volume, $\ln(v\varepsilon)$, and the elution volume, and thus the linear calibration function with constant P and slope Q . The third term on the right-hand side of eqn. 13 is the contribution to the elution volume, V_v , caused by the viscosity phenomenon in the interstitial volume, which is proportional to the difference in the viscosities of the polymer solution and the solvent (*i.e.*, the specific viscosity, η_{spec}). The proportionality constant, k' , is characteristic of the given separation system. With respect to the relatively complex form of eqn. 1, we can define constants A and B as

$$A = \frac{1}{\varepsilon_0} \quad (14)$$

$$B = \frac{1}{g_x} \left(\frac{\varepsilon_0 - 1}{\varepsilon_0} \right) \quad (15)$$

Although the definitions of A and B are different from those given in Part I¹, it allows us to use the same mathematics to solve eqn. 13. Thus, if we substitute eqns. 1, 10, 12, 14 and 15 and also the Huggins equation:

$$\eta_{\text{spec}} = g[\eta] + k_H[\eta]^2 g^2 \quad (16)$$

where $[\eta]$ is intrinsic viscosity and k_H is Huggins constant for the corresponding polymer-solvent system, into eqn. 13, we obtain

$$\begin{aligned} V_e = P + Q \ln v - \frac{Q}{L} \cdot \int_0^L \ln \left[A + \frac{Bg_I \sigma_I}{\sqrt{\sigma_I^2 + u \cdot \frac{\sigma_T^2 - \sigma_I^2}{L}}} \right] du + \\ + \frac{k'[\eta] g_I \sigma_I}{L} \cdot \int_0^L \frac{du}{\sqrt{\sigma_I^2 + u \cdot \frac{\sigma_T^2 - \sigma_I^2}{L}}} + \\ + \frac{k'k_H[\eta]^2 g_I^2 \sigma_I^2}{L} \cdot \int_0^L \frac{du}{\sigma_I^2 + u \cdot \frac{\sigma_T^2 - \sigma_I^2}{L}} \end{aligned} \quad (17)$$

By solving eqn. 17 we obtain

$$\begin{aligned} V_e = P + Q \left[\ln v + \frac{B^2 g_I^2 \sigma_I^2}{(\sigma_T^2 - \sigma_I^2) A^2} \cdot \ln \left(\frac{\sigma_T A + Bg_I \sigma_I}{\sigma_I A + Bg_I \sigma_I} \right) - \frac{Bg_I \sigma_I}{(\sigma_T + \sigma_I) A} + \right. \\ \left. \frac{\sigma_I^2}{\sigma_T^2 - \sigma_I^2} \cdot \ln(A + Bg_I) - \frac{\sigma_T^2}{\sigma_T^2 - \sigma_I^2} \cdot \ln \left(\frac{\sigma_T A + Bg_I \sigma_I}{\sigma_T} \right) \right] + \\ + k' \left(\frac{2[\eta] g_I \sigma_I}{\sigma_T + \sigma_I} + \frac{2k_H[\eta]^2 g_I^2 \sigma_I^2}{\sigma_T^2 - \sigma_I^2} \cdot \ln \frac{\sigma_T}{\sigma_I} \right) \end{aligned} \quad (18)$$

Some interesting conclusions follow from the solution of eqn. 18, which for $g_I = 0$ reduces to

$$V_{e0} = P + Q \ln(v\varepsilon_0) \quad (19)$$

Eqn. 19 is a linear calibration function which can be ascertained experimentally by extrapolating the concentration dependence of the elution volumes of standard polymer samples of various molecular weights to zero concentration. Eqn. 18 can be then used as a calibration function which also includes the effect of concentration. Eqn. 18 reduces to the form of eqn. 19 also for $\sigma_I = 0$, *i.e.*, for an infinitely small (zero) volume of injected solution. The dependence of the elution volume on concentration is also a function of the efficiency of the column system, as is obvious from eqn. 18. The efficiency can be evaluated from the contribution of separation columns to the total width of the elution curve, which is proportional to the difference $\sigma_T - \sigma_I$ or $\sigma_T^2 - \sigma_I^2$. For the limiting case of an infinitely high efficiency of the separation system, where $\sigma_T = \sigma_I$, eqn. 18 becomes

$$V_{e\infty} = P + Q \left(\ln v + \frac{Bg_I}{2A} + k'[\eta] g_I \right) \quad (20)$$

Using eqn. 18, we can establish the ratio of the contribution of the change in elution volume due to the changes in the hydrodynamic volume of the polymer coils (resulting from changes in concentration), to the contribution of viscosity phenomena in the interstitial volume. Let us to express this ratio by F . It follows from the above discussion that

$$F = \frac{V_e - V_{e0} - V_v}{V_v} \quad (21)$$

Substitution of eqns. 18 and 19 into eqn. 21 and rearrangement give

$$\begin{aligned} F = & \frac{Q}{k'} \cdot \left[\frac{B^2 g_I^2 \sigma_I^2}{A^2} \cdot \ln \left(\frac{\sigma_T A + Bg_I \sigma_I}{\sigma_I A + Bg_I \sigma_I} \right) - \frac{Bg_I \sigma_I (\sigma_T - \sigma_I)}{A} + \right. \\ & \left. + \sigma_I^2 \ln(A + Bg_I) - \sigma_T^2 \ln \left(\frac{\sigma_T A + Bg_I \sigma_I}{\sigma_T} \right) + (\sigma_T^2 - \sigma_I^2) \ln A \right] \times \\ & \times \left[2[\eta] g_I \sigma_I (\sigma_T - \sigma_I) + 2k_H [\eta]^2 g_I^2 \sigma_I^2 \ln \left(\frac{\sigma_T}{\sigma_I} \right) \right]^{-1} \quad (22) \end{aligned}$$

In Part II² we gave experimental evidence that the width of the elution curve is also a function of specific viscosity:

$$\sigma_T = f(\eta_{\text{spec}}) \quad (23)$$

which means that eqn. 10 does not hold precisely. Also, the shapes of elution curves and of concentration profiles are not Gaussian at higher concentrations but are con-

siderably deformed. However, we wished to ascertain in this study especially the relative contributions of individual factors to the overall concentration effect in the way in which they are formulated in eqns. 21 and 22. However, the above experimentally found deviations from theoretical assumptions, which form the basis for the derivation of all of the relationships, are compensated for in eqn. 22, because this equation reveals the ratio defined in eqn. 21 and not the absolute values of the elution volumes.

Moreover, in Parts II and III^{2,3} we showed that the use of statistical moments of elution curves, *i.e.*, of the mean elution volume and width calculated from variance instead of the maximum elution volume and width evaluated graphically, gives correct experimental results that are in agreement with theory also for distorted elution curves.

DISCUSSION

The analysis of eqn. 18 allows one to ascertain the contribution of its parameters and their variations to the changes in elution volumes with concentration. This analysis was carried out with some earlier experimental data¹⁻³. Most of the required values were calculated by means of expressions given in this paper and they are summarized in Table I. The values of B_1 and $B_{0.524}$ given there were calculated from eqns. 2-4, 6, 7 and 15 using the values $\Phi_x = 1$ and $\Phi_x = 0.524$ (see eqn. 6), respectively, *i.e.* the values which were discussed by Rudin and Wagner⁵. In calculations, we also used the Mark-Houwink equation for polystyrene in tetrahydrofuran:

$$[\eta] = 1.17 \cdot 10^{-2} M^{0.717} \quad (24)$$

which was referred to in preceding papers in this series, the Huggins constant $k_H = 0.362$ (see also refs. 1-3) and the values $\sigma_T = 1.75$ and $\sigma_I = 0.25$, which are the average experimental values taken from Part I¹. The values σ_T and σ_I were varied in order to ascertain the effect of this variation on the results calculated from eqns. 18 and 22.

TABLE I
MOLECULAR PARAMETERS OF POLYSTYRENE (PS) STANDARDS

Standard	$\bar{M}_w \cdot 10^{-3}$	$v \cdot 10^{18}$	A	B_1	$B_{0.524}$
PS 1	2610	462.5	0.2811	76.72	146.4
PS 3	867	88.55	0.3570	39.55	75.48
PS 4	498	38.55	0.4027	27.85	53.15
PS 6	200	9.811	0.4908	15.04	28.70
PS 8	98.2	3.375	0.5727	8.844	16.88

The dependence of the variation of V_e on g_I was determined first from eqn. 18, assuming that only the effect of the concentration expansion of the macromolecular coil in solution is of use, in such way that the values $P = 0$, $Q = -1$ and $k' = 0$

were employed (the choice of values for P and Q is arbitrary; the negative sign indicates that the slope of the function defined by eqn. 19 is negative). This procedure allows us to establish the principal course of the function V_e versus g_I under the chosen model conditions. The results are illustrated in Fig. 1.

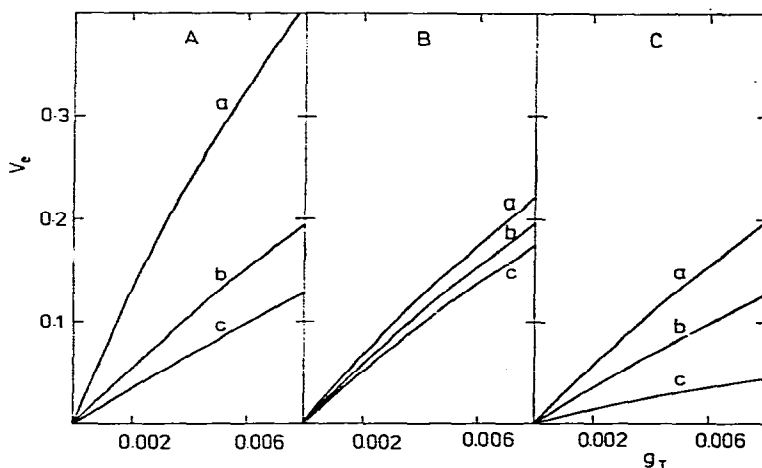


Fig. 1. Dependence of elution volume calculated from eqn. 18 ($P = 0$, $Q = -1$, $k' = 0$) on concentration. (A) Influence of molecular weight, $\sigma_T = 1.75$, $\sigma_I = 0.25$: (a) PS 1; (b) PS 3; (c) PS 4. (B) Influence of column efficiency, PS 3, $\sigma_I = 0.25$: (a) $\sigma_T = 1.5$; (b) $\sigma_T = 1.75$; (c) $\sigma_T = 2.0$. (C) Influence of injected volume, PS 3, $\sigma_T = 1.75$: (a) $\sigma_I = 0.25$; (b) $\sigma_I = 0.15$; (c) $\sigma_I = 0.05$.

The way in which the effect of molecular weight acts is obvious from Fig. 1A; the functions shown are increasing and convex. The increasing steepness of these functions with increasing molecular weight agrees with experimental observations. The effect of varying σ_T is shown in Fig. 1B. With increasing σ_T , *i.e.*, with decreasing column efficiency, the concentration effect becomes smaller, as can be seen from the decreasing steepness of the functions shown, which agrees with experimental observations³. The influence of changes in σ_I is shown in Fig. 1C. A decrease in σ_I is accompanied by a decrease in the steepness of the functions shown, which agrees with experimental observations¹.

Fig. 2 shows the courses of the functions calculated from eqn. 18 with the chosen values $P = 0$, $Q = 0$ and $k' = 1$, *i.e.*, the dependence of elution volume on concentration was investigated for the cases when only the viscosity phenomena in the interstitial volume are operative.

Almost identical statements can be made about the effect of molecular weights, σ_T and σ_I , on the course of the examined functions with the exception that all of the functions are concave.

It should be possible to plot $V_e = f(\sigma_T)$, $V_e = f'(\sigma_I)$ functions, etc., if the other corresponding parameters in eqn. 18 are assumed to be constant. However, the course of these functions is obvious from eqn. 18 and from Figs. 1 and 2.

The ratio of the change in elution volume caused by the concentration expansion of macromolecules in the pores to the change caused by viscosity phenomena was calculated by means of eqn. 22. The calculation was carried out for polystyrene

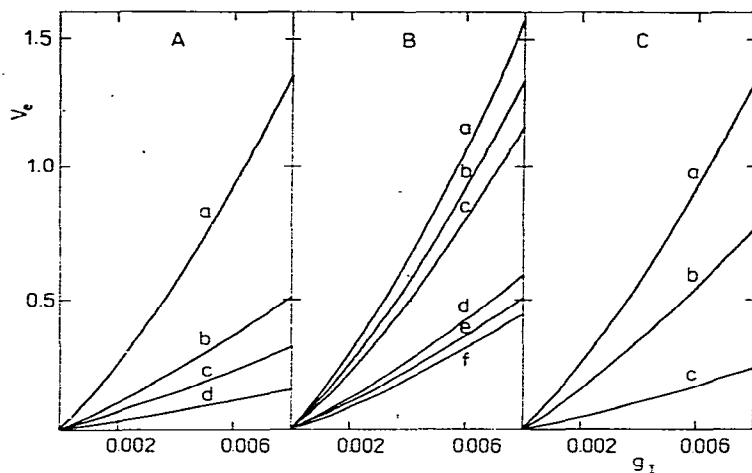


Fig. 2. Dependence of elution volume calculated from eqn. 18 ($P = 0$, $Q = 0$, $k' = 1$) on concentration. (A) Influence of molecular weight, $\sigma_T = 1.75$, $\sigma_I = 0.25$: (a) PS 1; (b) PS 3; (c) PS 4; (d) PS 6. (B) Influence of column efficiency, $\sigma_I = 0.25$: (a) PS 1, $\sigma_T = 1.5$; (b) PS 1, $\sigma_T = 1.75$; (c) PS 1, $\sigma_T = 2.0$; (d) PS 3, $\sigma_T = 1.5$; (e) PS 3, $\sigma_T = 1.75$; (f) PS 3, $\sigma_T = 2.0$. (C) Influence of volume injected, PS 1, $\sigma_T = 1.75$: (a) $\sigma_I = 0.25$; (b) $\sigma_I = 0.15$; (c) $\sigma_I = 0.05$.

standards¹, the elution volumes of which are in the middle linear part of the calibration function defined by eqn. 19 and shown in Fig. 3. Calculated values of F (see eqn. 22) are given in Tables II and III for $Q = -1$ and $k' = 1$ and concentrations g_I varying in the range 0.8–0.0125% (w/v) of various polystyrene standards for various σ_T , σ_I and Φ_x values. It follows from Table II that F (for $Q = -1$ and $k' = 1$) increases moderately with decreasing concentration for various molecular weights of polystyrene standards from $F = 0.37$ to 0.52. These values of F hold for the given values of σ_T and σ_I and $\Phi_x = 1$. The F values are approximately double when $\Phi_x = 0.524$.

The effect of variation of σ_T and σ_I on F can be seen from Table III. F is only slightly sensitive to changes in these parameters and its value lie in the above-mentioned region. The resulting values of F also depend on Q and k' . These values were $Q_1 = -3.01$, $k'_1 = 7.957$ and $Q_2 = -2.36$, $k'_2 = 6.754$ for the two experimental systems studied in Part III³.

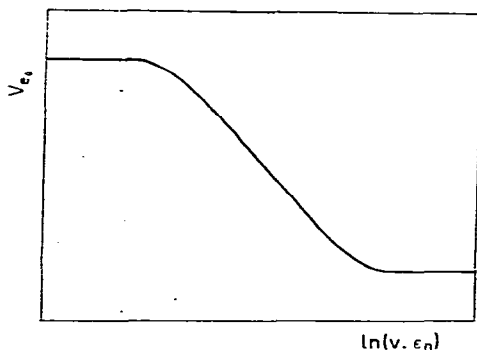


Fig. 3. Calibration graph for hypothetical separation system.

TABLE II

INFLUENCE OF CONCENTRATION, MOLECULAR WEIGHT AND CRITICAL VOLUME FRACTION ON THE VALUE OF F ($Q = -1$, $k' = 1$) FROM EQN. 22

Other data used	Concentration, g_1 (%, w/v)	F		
		PS 3	PS 4	PS 6
$\sigma_I = 0.25$, $\sigma_T = 1.75$, $\Phi_x = 1$	0.8	0.3858	0.3953	0.3730
	0.4	0.4455	0.4366	0.3927
	0.2	0.4817	0.4601	0.4033
	0.1	0.5017	0.4728	0.4087
	0.05	0.5123	0.4793	0.4115
	0.025	0.5177	0.4826	0.4129
	0.0125	0.5205	0.4843	0.4136
$\sigma_I = 0.25$, $\sigma_T = 0.75$, $\Phi_x = 0.524$	0.8	0.6735		0.6897
	0.2	0.8931		0.7629
	0.05	0.9700		0.7835
	0.0125	0.9914		0.7888

The resulting values of F lie in the range 0.13–0.20, showing that the viscosity phenomena in the interstitial volume contribute decisively to the overall concentration effect, *i.e.*, to the extent of about 80–87% under the real experimental conditions considered. This contribution, of course, is valid only for that part of the calibration function where the given value of Q holds. In the two extreme regions of the calibration graph where curvature occurs (see Fig. 3), the Q decreases and, consequently, the contribution of viscosity phenomena increases, and in the two end regions (outside the separation range) where $Q = 0$ the viscosity contribution to the concentration effects is 100%.

TABLE III

INFLUENCE OF CONCENTRATION, EFFICIENCY OF SEPARATION SYSTEM AND VOLUME INJECTED ON THE VALUE OF F ($Q = -1$, $k' = 1$) FROM EQN. 22

Other data used	Concentration, g_1 (%, w/v)	F (PS 4)
$\sigma_I = 0.25$, $\sigma_T = 1.5$, $\Phi_x = 1$	0.8	0.3873
	0.2	0.4576
	0.05	0.4786
	0.0125	0.4842
$\sigma_I = 0.25$, $\sigma_T = 2.0$, $\Phi_x = 1$	0.8	0.4019
	0.2	0.4622
	0.05	0.4799
	0.0125	0.4845
$\sigma_I = 0.15$, $\sigma_T = 1.75$, $\Phi_x = 1$	0.8	0.4188
	0.2	0.4674
	0.05	0.4812
	0.0125	0.4849
$\sigma_I = 0.05$, $\sigma_T = 1.75$, $\Phi_x = 1$	0.8	0.4536
	0.2	0.4774
	0.05	0.4839
	0.0125	0.4857

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