Estimation of thermodynamic quality of the solvent from the concentration effect in gel permeation chromatography of polymers

T. Bleha, D. Bakoš and D. Berek
Polymer Institute, Slovak Academy of Sciences, 809 34 Bratislava, Czechoslovakia
(Received 26 April 1977)

Dependences of the elution volume, V_e, on the concentration of the injected polystyrene (PS), c, in gel chromatography with inorganic carriers were studied in various single and binary eluents. It was found that the dependence of V_e on c is approximately linear in the region of low concentrations and its slope depends on the thermodynamic quality of the eluent. This relationship is more pronounced for higher molecular weights. Correlation for the slope k with the limiting viscosity numbers $[\eta]$ proposed earlier in the literature is not valid for thermodynamically poor eluents; however, a correlation was found for all the eluents employed between k and the product A_2M, where A_2 is the second virial coefficient of polymer in solution. This supports the interpretation of the concentration effect in gel chromatography of polymers as a consequence of the decreasing effective hydrodynamic volume of coils with increasing concentration. The link between the empirically found correlation of k with A_2M and the existing theory of the concentration effect in g.p.c. was examined. Comparison of the virial coefficients determined from the slope k for PS in various eluents with the literature data showed that gel chromatography could yield a rapid estimate of the value A_2. As has been shown by an analysis of the concentration effect on the distribution coefficient K the concentration effect leads to the formation of a non-linear isotherm and to the asymmetrical peak shapes. In the range of very low concentrations, deviations from the linear dependence of V_e on c can be expected as a result of both the sorption effect and the thermodynamic partition of the solute.

INTRODUCTION

The effect of the concentration of the injected polymer, c on the shift of peak elution volume, V_e, in g.p.c. designated as concentration effect is one of several operational variables affecting V_e of the solute. In its consequences, the concentration effect complicates the determination of polymer molecular weight and its distribution function by g.p.c. It is therefore recommended to work at the lowest concentrations of the injected polymer or to extrapolate the results to zero concentration[1–4]. The concentration effect does not depend on the type of gel used and is more marked for polymers with narrow distribution curve compared to polydisperse samples. Originally the reason for the concentration phenomenon in g.p.c. of synthetic polymers was looked for in viscosity effects in the system. A correlation was found between the logarithm of the slope k of the linear dependence of V_e on c and the logarithm of limiting viscosity numbers $[\eta]$[5], but also directly between k and $[\eta]$[6]. It was only later that the concentration effect of polymers in g.p.c. was explained as a consequence of the reduction of the effective hydrodynamic volume of the flexible polymer coils with an increase of their concentration in solution[7–9]. With regard to the primary mechanism of the g.p.c. separation (steric exclusion) the decrease of effective hydrodynamic volume will manifest itself by an increase of V_e.

So far the concentration effect has been observed for many synthetic polymers especially if thermodynamically good solvents were used. In previous papers[4,10–12] it has been shown that as the thermodynamic quality of eluent deteriorates, the concentration effect decreases. For eluents representing θ-solvents for the given polymers, the concentration effect practically disappears; therefore the use of θ-eluents is advantageous from this viewpoint. The systematic study of the concentration effect and its dependences on the quality of eluent is made possible by working with binary eluents of the type solvent–precipitant. Such binary eluents with variable content of a precipitant have already been employed in the study of adsorption and partition effects in g.p.c.[10,13].

In the present paper we concentrate on the more quantitative treatment of the link between the slope k of the concentration dependence and the quality of the eluent. It will be shown that the concentration dependence in gel chromatography could be used for estimating A_2, the second virial coefficient of the system injected polymer–eluent.

EXPERIMENTAL

G.p.c. apparatus and the compounds used have been described in more detail[10,13]. A two column system was packed

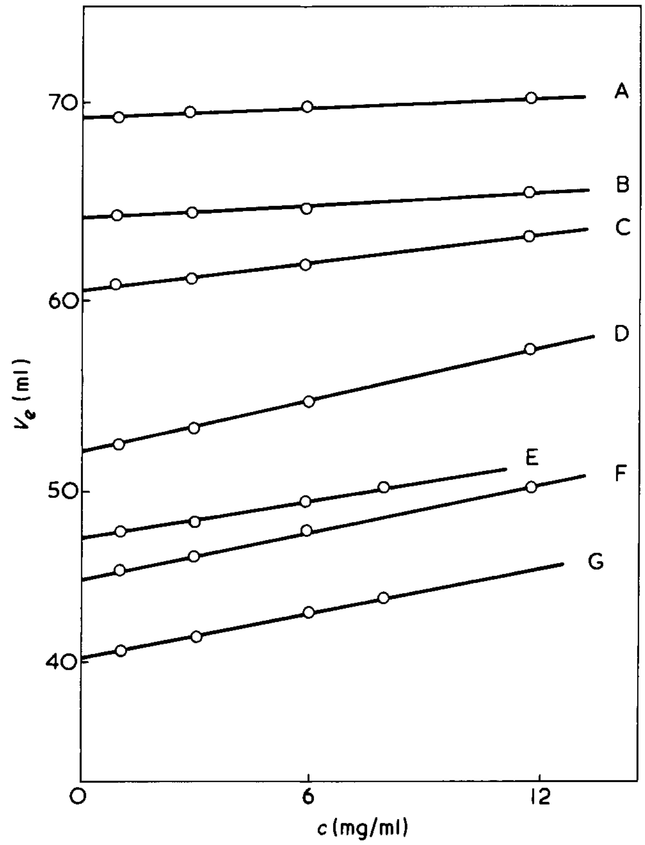

Figure 1 Concentration dependences of elution volume for PS in benzene–methanol mixture (90:10) M_v: A, 51 000; B, 97 200; C, 160 000; D, 320 000; E, 498 000; F, 670 000; G, 1.2 × 10^6

with Porasil D and Porasil E separated approximately within the molecular weight region 5 x 10^4 and 5 x 10^6. A differential refractometer Knauer, Model 2025 (Knauer, West Berlin) was used as detector and the elution behaviour of PS standards (Pressure Co., Pittsburgh, USA) was studied in the molecular weight range 5.1 x 10^4 and 1.8 x 10^6. In addition to eluents employed earlier[10], i.e. benzene, chloroform, tetrahydrofuran, butanone-2, mixtures (v/v) of benzene–methanol (77.8: 22.2), chloroform–methanol (74.7:25.3) and butanone-2/n-heptane (50:50), concentration dependences of V_e were studied for PS in mixtures of benzene–methanol and methanol–chloroform (90:10) and in a mixture of butanone and n-heptane (68.4:31.6). The thermodynamic quality of these mixtures for PS is between that of good single eluents and θ-eluents. The experiment was performed either at the ambient temperature or at a temperature of 25° ± 0.2°C. Thermodynamic parameters for all the eluents used are summarized in ref 13. Apart from this, elution volumes of PS were examined in benzene and in benzene–methanol mixtures (77.8:22.2 vol % θ-mixture at 25°C) in such a way that the columns were thermostatted at 60°C, while the siphon system recording the volume of the eluate was at ambient temperature. By comparing these results with the data obtained previously for the ambient temperature[10] the influence of temperature on the concentration effect can be evaluated.

RESULTS AND DISCUSSION

Estimation of the virial coefficient A_2 *from the slope* k

Figures 1–3 present the concentration dependences of V_e for PS in three thermodynamically intermediate solvents. Their course shows that similarly as for the majority of the cases studied earlier, in the low concentration region of the injected polymer $V_e(c) = V_e(0) + kc$ holds and a linear extrapolation of V_e to the infinitely diluted solution can be used. When comparing the slopes of concentration dependences k in *Figures 1–3* with the k values measured previously[10], it is seen that the former lie between the values for single and θ-solvents as expected. Thereby they increase with molecular weight and reach the maximum approximately for a sample 6.7 x 10^5 analogously as observed in single eluents. A decrease in the slopes after the maximum might be attributed to less effective separation of polymer in the high molecular region. The proper choice of distributions of pores (gel type) should shift the maximum of slopes to high molecular range or, the maximum need not exist. For macromolecules excluded from gel pores, the slope approaches zero.

The thermodynamic quality of eluents can be changed not only by adding precipitant to single eluent but also by temperature variation. Therefore concentration dependences of PS were examined in benzene and in a benzene–methanol (77.8:22.2) mixture at elevated temperature by heating the columns to 60°C. Results for three molecular weights are given in *Figure 4*. For simplicity, a straight line was plotted through experimental points obtained for PS in benzene at 25°C, though the plots V_e–c in *Figure 1* in our previous paper[10] seemed to be slightly curved. Comparison of the concentration dependences at both temperatures shows that the thermodynamic quality of benzene for PS becomes worse with rise of temperature, since the slopes k decrease. It is reversed with eluent mixture; the mixture represents θ-solvent at 25°C (V_e is practically independent of c), but at 60°C the concentration dependences become relatively distinct, i.e. the thermodynamic quality of mixed eluent will improve substantially. These results can be compared with the A_2

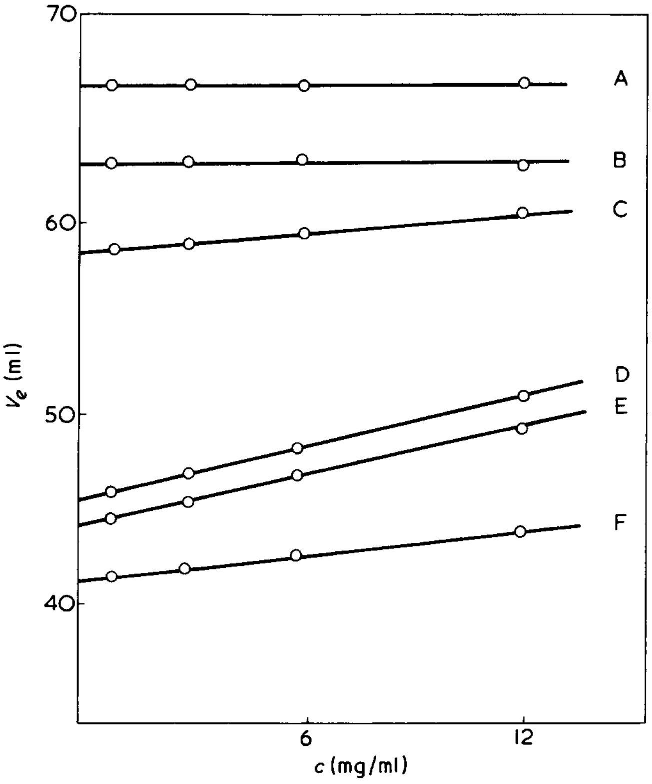

Figure 2 Concentration dependences of elution volume for PS in chloroform–methanol mixture (90:10) M_v: A, 51 000; B, 97 000; C, 160 000; D, 498 000; E, 670 000; F, 1.2 × 10^6

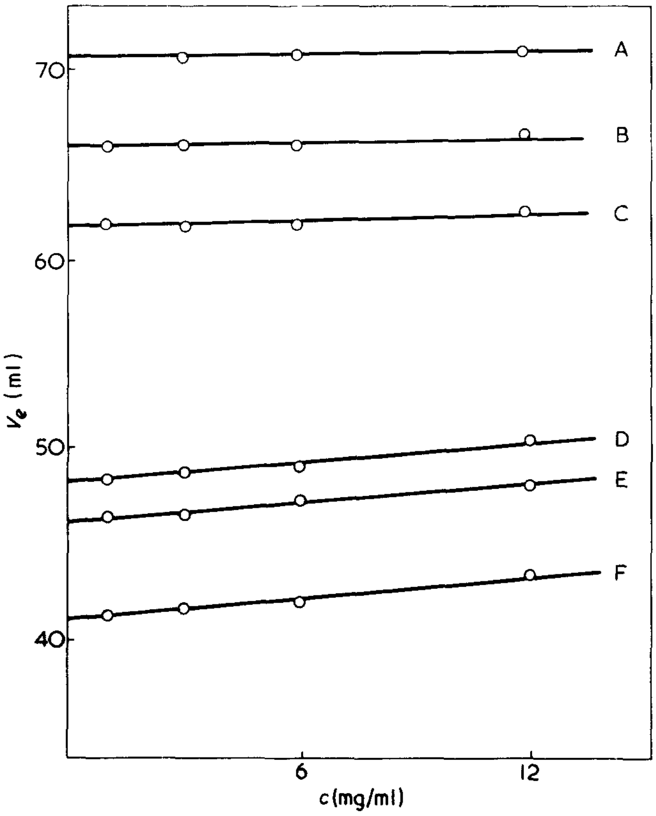

Figure 3 Concentration dependences of elution volume for PS in butanone-2–n-heptane (68.4:31.6) M_v: A, 51 000; B, 97 000; C, 160 000; D, 498 000; E, 670 000; F, 1.2 X 10^6

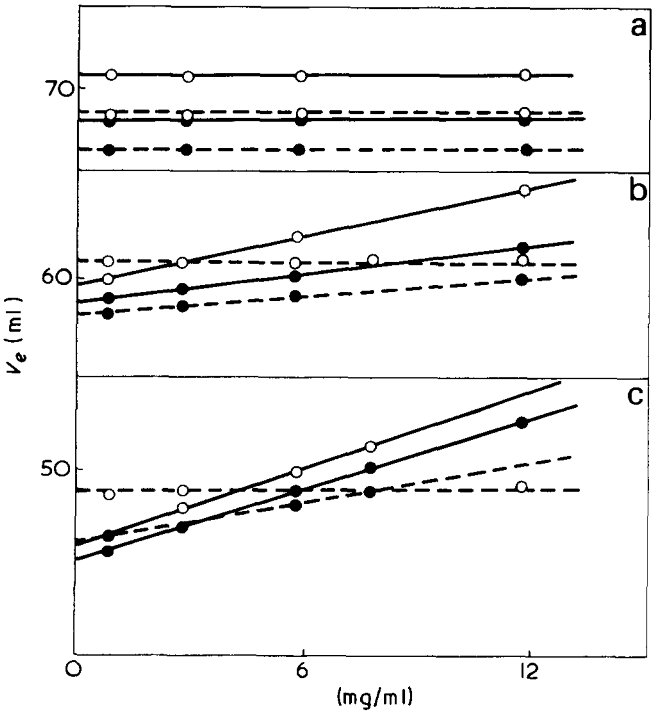

Figure 4 Concentration dependences of elution volume for PS in benzene (———) and benzene–methanol (— — — —) mixture (77.8: 22.2) at 25° (○) and 60°C (●): (a) 51 000; (b) 160 000; (c) 498 000

values obtained from the light scattering measurements for PS with $M = 1.75 \times 10^6$ in benzene and its mixtures with ethanol at 25° and 60°C[14]. It was found that $dA_2/dT <$ 0 for pure benzene and similarly the mixture containing 20% ethanol becomes thermodynamically poorer for PS (A_2 changes from 1.75 at 25°C to 1.55 cm^{3}mol/g^2 x 10^{-4} at 60°C). On the other hand, the benzene–ethanol mixture containing 30% of ethanol (θ-mixture for PS at 25°C) showed an increase of A_2 from 0 to 1.1 cm^3 mol/g^2 x 10^{-4} when the temperature was raised from 25° to 60°C.

According to previous proposals[6,7] we tried to correlate the values of the slopes of concentration dependences k (within the range of effective separation, i.e. to M about 6.7 x 10^5) with the limiting viscosity numbers $[\eta]$ of the injected polymer in given eluents studied so far in our laboratory[10]. *Figure 5* shows the dependences obtained, whereby the linear or logarithmic plots of both quantities were chosen. The $[\eta]$ values were either found experimentally[13] or calculated from the relation $[\eta] = KM^a$. Constants a and K were taken from the literature and are listed in ref 13. From

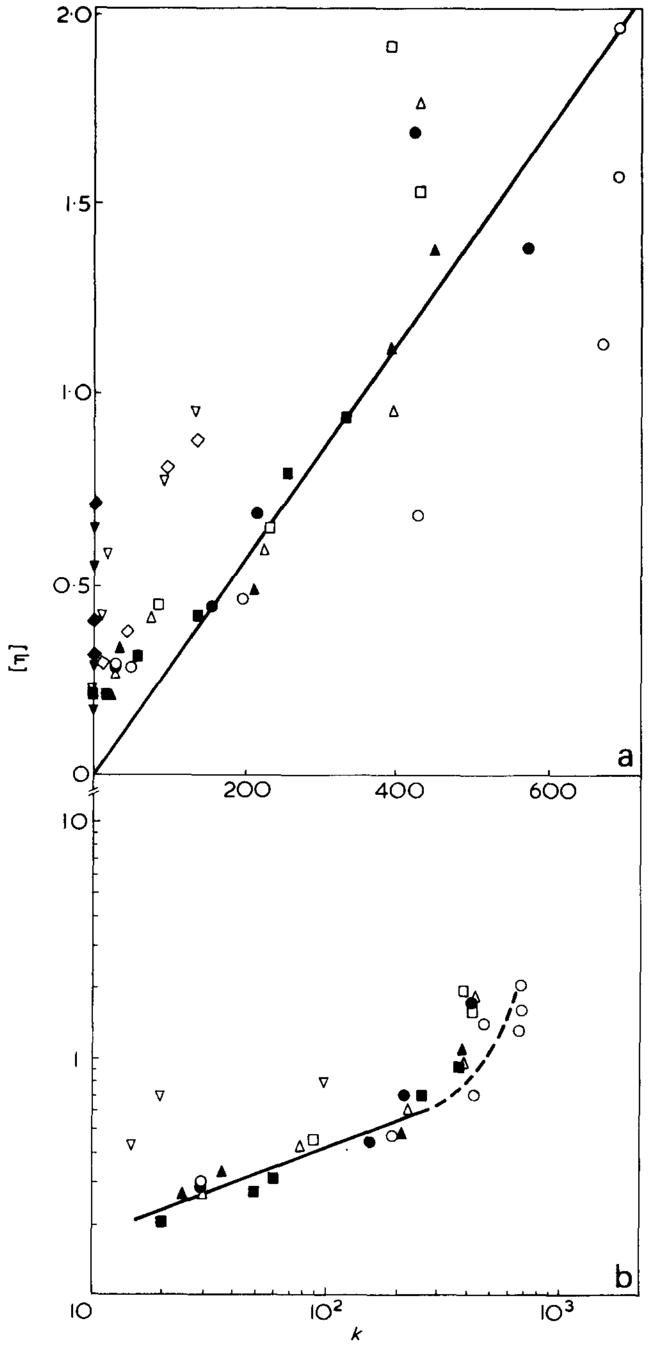

Figure 5 (a) Plot of the slope k against limiting viscosity number $[\eta]$. (b) Plot of log k against log $[\eta]$. ○, B; ●, THF; □, CH; ■, MEK, △, B–M; ▲, CH–M; ◇, MEK–H; ▽, B–M (theta); ▼, CH–M (theta); ◆, MEK–H (1:1).

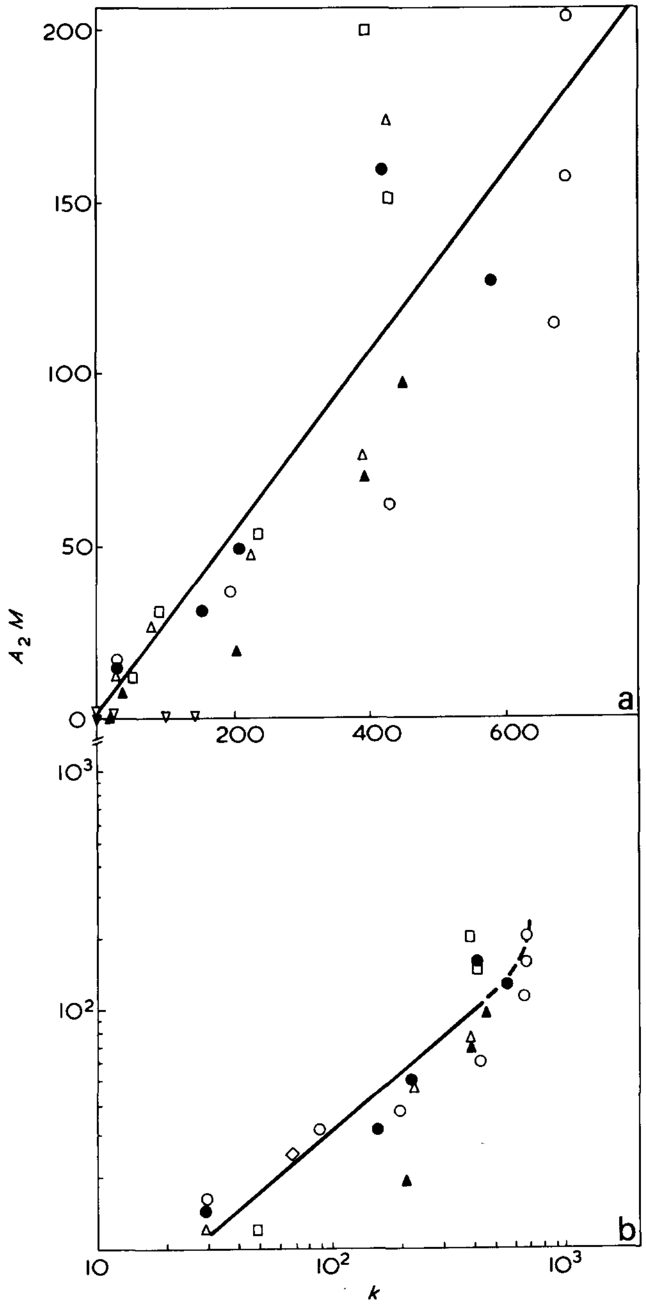

Figure 6 (a) Plot of A_2M against k. (b) Plot of log A_2M against log k. The points are denoted as in *Figure 5*.

Figure 5a it follows that for good and intermediately good solvents it is possible to consider the slope k as being approximately directly proportional to $[\eta]$, whereby the dependence $k-[\eta]$ does not pass through the origin. A marked deviation from linear dependence is manifested in *Figure 5a* in two regions. In the first region, for good solvents (especially $CHCl_3$) the slope seems to be increasing slower than the corresponding value $[\eta]$ which is probably connected with the mentioned decrease of the slopes at higher molecular weights due to gel properties. In the second region, at low k values where all the used θ-mixtures are situated – there is no correlation between k and $[\eta]$. Similarly the correlation between the logarithms k and $[\eta]$ in *Figure 5b* is not linear in the good solvents studied.

Although the limiting viscosity number $[\eta]$ is not the most proper measure of the thermodynamic quality of eluent, correlation in *Figure 5* points out that the extent of interaction between polymer and eluent can be estimated from the measurements of concentration dependences in g.p.c. A more suitable parameter for quantitative description of these interactions appears to be the second virial coefficient A_2. We did not find sufficient literature data of A_2 values for PS in the solvents used in the present work. Therefore we determined the product A_2M from $[\eta]$, employing correlation between $A_2M/[\eta]$ and $\alpha_s^2 - 1$ found for PS as well as other polymers in solvents of various quality[15,16]. The expansion factor of the radius of gyration is connected to the viscosity expansion factor $\alpha_\eta = ([\eta]/[\eta]_\theta)^{1/3}$ (where $[\eta]_\theta$ is the limiting viscosity number in θ-solvent) by the relation $\alpha_\eta^3 = \alpha_s^{2.43}$ [15]. For good solvents, the ratio $A_2M[\eta]$ is almost constant and close to 100, while it decreases as the quality of solvent becomes worse so as A_2 approaches zero[15,16]. By using this correlation we plotted A_2M against k (*Figure 6a*). The points lie approximately on straight lines passing through the origin, the scatter of points being relatively large mainly in the range of high values of A_2M. Similarly, in *Figure 6b* log A_2M is plotted against log k, the dependence being almost linear as well.

It follows from *Figures 6a* and *6b* that the slope of concentration dependence of PS in g.p.c. on inorganic carriers of the Porasil type within the range of 1 order of magnitude of molecular weights between 5×10^4 and about 6×10^5 is a function of the quality of the solvent expressed by virial coefficient A_2. To solve the problem whether this correlation is indeed linear as illustrated in *Figure 6a* and whether it is better to be expressed by log k and log A_2M as shown in *Figure 6b*, more exact experiments are needed.

One can assume that similar variation of A_2M with k should be valid also for other polymers as well as various inorganic or organic gels in the domain of an effective separation. This would enable the estimation from the known value of A_2, the magnitude of the concentration effect or to make corresponding corrections of g.p.c. results.

A much more interesting phenomenon is the reverse procedure of determining the virial coefficient for the injected polymer in eluent from the slope k. In studying this procedure the following preliminary results were obtained for PS in toluene. For molecular weights of PS 160 000 and 498 000 A_2M were read from linear dependence in *Figure 6a* for measured k values and thus the values 4.25 and 2.3 cm^3 $mol/g^2 \times 10^{-4}$ were obtained. For comparison by interpolation of the dependence of A_2 on M for system PS–toluene plotted from values obtained by light scattering[17] for the two mentioned molecular weights, $A_2 = 4.5 \times 10^{-4}$ cm^3 mol/g^2 and $A_2 = 3.4 \times 10^{-4}$ cm^3 mol/g^2 which is in good agreement with our data obtained by means of g.p.c. It appears that the hydrodynamic effects on the concentration dependence of elution volume do not play a significant role in g.p.c.; the system is in quasi-thermodynamic equilibrium which makes possible the determination of thermodynamic quantities for diluted solution of the injected polymer

Linear correlation between A_2M and k can be justified to some extent also on the basis of the following considerations. For good solvents coefficient A_2 is proportional to molecular weight according to the relation $A_2 \simeq M^{-v}$, where v equals approximately the difference $1 - a$, a being the exponent of relation $[\eta] = KM^a$. Then also $A_2M \simeq M^a$ is confirmed by the constant value of ratio $A_2M/[\eta]$ close to 100 for good solvents[15]. As the A_2 coefficient decreases with molecular weight, A_2M increases with increasing k. In other words, both parameters A_2M and k have the same molecular weight dependence. The scatter of points in *Figure 6* shows that this dependence is not elaborated quantitatively though qualitative features of correlation between A_2M and k are

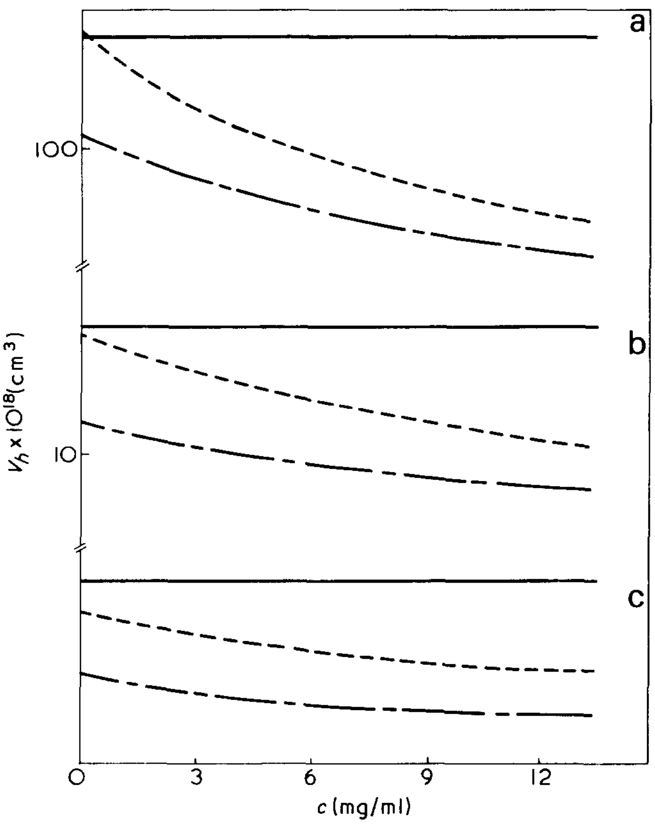

Figure 7 Variation of the logarithm of the hydrodynamic volume with *c* of injected PS for benzene and θ-mixture benzene–methanol (77.8:22.2) calculated according to equation (1) (— — — —), (— - — -) and equation (3) (———). (a) 1.2 × 10^6; (b) 320 000; (c) 97 100

possible. Similarly as in the measurement of light scattering or osmotic pressure of solution, the estimate of a virial coefficient from g.p.c. concentration effect is based on extrapolation of the experimental points to an infinitely diluted solution. The method is fast and the sample consumption is small. The main advantage consists, however, in the possibility of determining the thermodynamic parameter A_2 of polymer within routine analytical chromatographic procedure.

Connection of correlation between A_2M *and* k *with the theory of concentration effect in g.p.c.*

The empirically found correlation between A_2M and k can be accounted for by the theory of the concentration effect in g.p.c. A semiempirical model depicting quantitatively the concentration effect in g.p.c. was proposed by Rudin[8]. His model stemmed from the idea that in the low concentration region the effective hydrodynamic volume of a polymer coil V_e decreases with increasing concentration of polymer in solution. The dependence of V_h on c as proposed by Rudin can be expressed after modification as:

$$V_h = M/(a_1 + a_2c) \qquad (1)$$

where a_1 and a_2 can be expressed by parameters available, *a priori* for both polymer and eluent. Rudin used successfully the model for explaining the concentration dependences for various polymer–solvent systems[8] as well as for determining universal calibration log V_h – V_e provided the hydrodynamic volume of solvated macromolecules V_h was determined at finite concentrations[9]. As has already been indicated[4,18], the effect of the quality of eluent is not included in the model in a proper way and thus the theory provides correct predictions of the concentration effect only for good solvents. It is well illustrated in *Figure* 7 showing the dependences of log V_h on the concentration of the injected polymer, *c*, for benzene and θ-mixture of benzene and methanol at 25°C calculated according to equation (1). In accord with the Rudin model[8,9] it was assumed that the polymer concentration in a column is equal to the injected concentration *c*. Despite the fact that for θ-mixture the effective hydrodynamic volume of solvated coil in the unperturbed state should be independent of *c*, calculation afforded the dependence of log V_h on *c* also in this case. If we further assume that in the middle region of universal calibration an equation:

$$\log V_h = (a - b)V_e \qquad (2)$$

is valid, then the linear increase of V_e with *c* observed experimentally should be manifested as a linear decrease of log V_h with *c*. But it is evident from *Figure* 7 that the curves already deviate from linearity at not too high concentrations used in g.p.c. even at lower molecular weights.

Rudin and Wagner[19] have very recently presented a modified model of the concentration effect to remove its deficiencies. According to this more rigorous model V_h is given by:

$$V_h = \frac{[\eta]M}{(9.3/4\pi) \times 10^{24} + N_0 \times c([\eta] - [\eta]_\theta)} \qquad (3)$$

with *c* in g/cm^3 and $[\eta]$ in cm^3/g, N_0 being Avogadro's constant. Comparison with the experimental data from the literature has shown that the points obtained at various concentrations in a good and in a theta solvent lie on a common curve of log V_h against V_e.

The new model of the effect of concentration has removed deficiency of the previous theory: as it is obvious from equation (3) at first sight, in the θ-mixtures V_h is independent of concentration. This is illustrated in *Figure* 7 for PS in θ-mixtures of benzene and methanol at 25°C as a full line. It should be mentioned, however, that the hydrodynamic volume at infinite dilution calculated according to equation (3) is for all cases equal approximately to double the values calculated by the original model (equation (1)). From the expression of V_h according to equation (3) it readily follows that also mutual connection exists between calibration of log V_h against V_e[9] and universal calibration of log $[\eta]M$ vs. V_e[20]. Both procedures are equivalent for an infinitely diluted solution of the injected polymer or for an eluent representing θ-mixture for the injected polymer. In other cases the plot of log V_h against V_e compared with the calibration of log $[\eta]$ M vs. V_e is always shifted to lower V_e.

Assuming for simplification the validity of equation (2) and substituting into equation (3), the slope of concentation dependence can be expressed as:

$$k = \frac{1}{2.303b} \times \frac{[\eta] - [\eta]_\theta}{(9.3/4\pi N_0) \times 10^{24} + c([\eta] - [\eta]_\theta)} \qquad (4)$$

As seen from equation (4), the revised theoretical model of the concentration effect[19] predicts the concentration dependent slope *k*, the value of which decreases with increasing *c*.

Relation (4) explains why the correlation of the slopes *k* with the limiting viscosity number $[\eta]$ (*Figure 5a*) deviates from the straight line in the range of low *k* values and why it

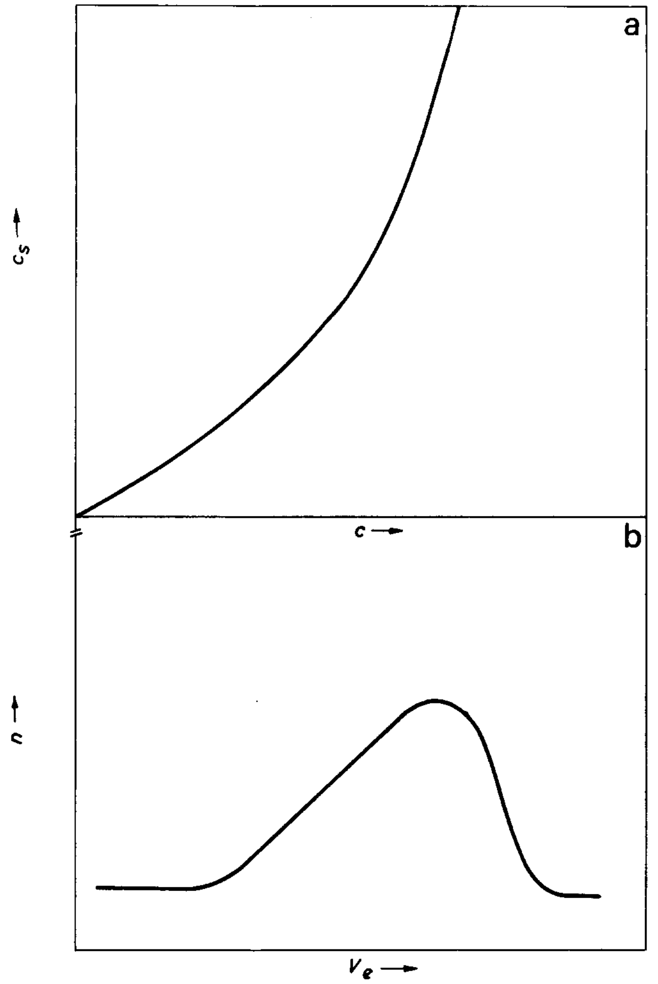

Figure 8 Schematic representation of the non-linear isotherm as a result of a decrease in the effective hydrodynamic volume of coils with concentration (a) and the corresponding shape of chromatographic peak (b)

does not pass through the origin. It also indicates that it would be more suitable to plot k against the difference $[\eta] - [\eta]_\theta$. This type of correlation is implicitly included in the plot of k vs A_2M (*Figure 6a*) as documented by a semi-empirical equation for calculating A_2 proposed by Krigbaum[21]:

$$[\eta] - [\eta]_\theta = 5.0 \times 10^{-3} A_2 M \qquad (5)$$

Discussion about the theory of the concentration effect can be concluded by the statement that the modified theoretical model of Rudin and Wagner[19] supports the possibility of employing the slope k for estimation of the thermodynamic interaction parameters for the polymer–eluent system.

The exact description of the concentration effect in g.p.c. should be, however, based on the rigorous theoretical model. Several relations expressing dependence of the expansion coefficient α[15,22,23] or the hydrodynamic volume V_h [24] on the polymer concentration in solution were derived for diluted solutions. In all approaches, the compression of a polymer coil with increasing c is proportional to the product A_2M. Application of these expressions to the analysis of the effect of concentration in g.p.c. just investigated in our laboratory should provide reliable theoretical justification for an empirically found method of estimating the second virial coefficients from g.p.c. measurements.

Effect of concentration on distribution coefficient K

Theoretical views on a decrease of the effective hydrodynamic volume of polymer coils with increasing concentration, which is the reason for the concentration effect in g.p.c. would be justified if the incompressible inorganic gels are used. The same concentration effect as e.g. in *Figures 1–3*, viz. an increase in the elution volume with concentration was observed also for soft organic gels of the Sephadex type[25–27]. The origin of the concentration effect is, however, in this case slightly different. Both types of gel can be compared by examining the concentration effect on the distribution coefficient K. The distribution coefficient is determined as a ratio of concentrations in the stationary and mobile phase $K = c_s/c$ and is connected with the elution volume by:

$$K_d = \frac{V_e - V_0}{V_i} \text{ or } K_{av} = \frac{V_e - V_0}{V_s} \qquad (6)$$

where V_0 is the excluded volume (the volume of solvent between gel particles), V_i is the inner volume of gel pores, and V_s is the total volume of the stationary phase given as a sum of V_i and the volume of gel matrix, V_m. The concentration in the mobile phase is identical with the concentration of the injected polymer only at the top of the column. Assuming a linear increase of V_e with c for the distribution coefficient at finite concentration $K_d(c)$: in the case of inorganic carriers we obtain from equation (6):

$$K_d(c) = K_d(0)\,\frac{V_e(0)(1 + k'c) - V_0}{V_e(0) - V_0} = K_d(0)(1 + \delta c) \qquad (7)$$

where $\delta = V_e(0)k'/(V_e(0) - V_0)$ is expressed by elution volume extrapolated to zero concentrations.

Since at finite concentrations all parameters on the right hand side of this equation are positive, the ratio $K_d(c)/K_d(0)$ is always greater than 1. This means that as c increases, the linear isotherm (relation between the concentration in the mobile and stationary phase) is deformed and the stationary phase is relatively enriched because the concentration in the stationary phase, c_s, can be expressed from equation (7) by virial expansion:

$$c_s = K_d(0) \times c + K_d(0)\delta c^2 \qquad (8)$$

The resulting concave isotherm is illustrated schematically in *Figure 8a* together with a corresponding typically deformed chromatographic peak (*Figure 8b*).

It follows from equations (7) and (8) that whenever $k' \neq 0$, i.e. if the effective hydrodynamic volume of coils changes and the concentration dependence of V_e is observed, the separation process is controlled by a non-linear isotherm. This complicates the interpretation of the g.p.c. chromatograms and necessitates the correction of the molecular weight distribution obtained by g.p.c. Usually, the necessary corrections for concentration effects are evaluated separately but there has already been proposed a method for the simultaneous evaluation of corrections for concentration effects and instrumental spreading in g.p.c.[28].

In the case of compressible organic gels, osmotic shrinkage (deswelling) of gel particles occurs as the concentration of solute increases, leading to a change of the inner volume V_i as well as V_s. Consequently this effect leads to an increase of V_e with increasing concentation of the injected polymer which complicates the study of associations in biologically interesting systems[25–27]. Baghurst *et al.*[27] evaluated the

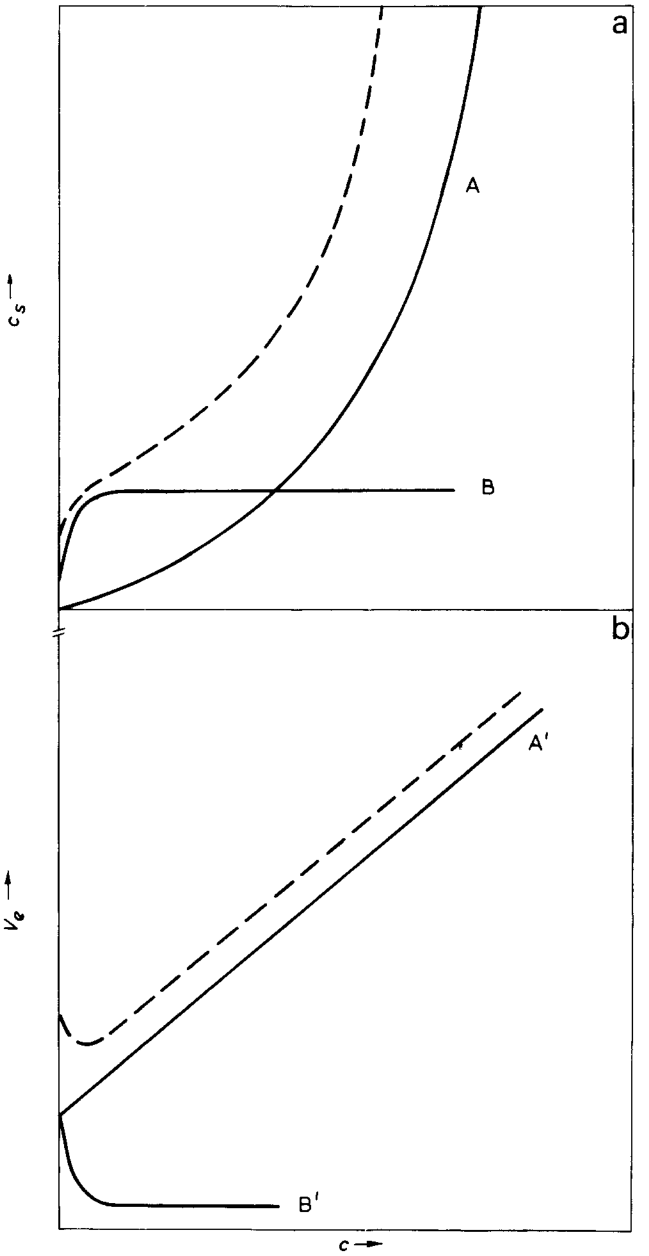

Figure 9 (a) Schematic representation of sorption isotherm (*S*), partition isotherm (*P*) (in Langmuir shape form), isotherm of steric exclusion (*SE* – curve from *Figure 8a* and resulting isotherm (– – –) of the separation process in binary eluents: A, *SE;* B, *S,P.* (b) Superposition of the individual contributions to the resulting concentration effect in binary eluents (– – – –): A, A', *SE;* B, B', *S, P*

effect of deswelling quantitatively for gels of the Sephadex type in aqueous solution. These authors expressed the slope k by using the virial coefficients for the pairs solute–eluent and gel–eluent. They assumed that neither V_0 nor K_{av} changed with c. The distribution coefficient, K_{av} can indeed be considered as constant for globular macromolecules since it is assumed that their size does not change with increasing concentration in dilute solutions. However, in the case of flexible polymer coils the reduction of their effective hydrodynamic volume will necessarily be manifested in the dependence of K_{av} or K_d on c even in the case of organic gels. But this relation will differ from equation (7), where we assumed constant inner volume V_i. Theoretical evaluation of the concentration effect on Sephadex according to ref 27 is thus not applicable to particles, the hydrodynamic volumes of which vary in solution with concentration. The resulting concentration dependence of V_e is in this case determined by a sum of both contributions: by a change of the inner volume of the gel and by the dependence of K on the concentration of injected solute.

The mechanism of g.p.c. separation determining the coefficients K_d or K_{av} has not been discussed in this paper. In general the steric exclusion is assumed to be a most important mechanism of the separation. It was shown earlier[10,13] that by using mixed eluents a contribution of sorption and partitioning of solute to the separation mechanism in g.p.c. must not be neglected. Now we shall try to show in greater detail the influence of these contributions to the concentration dependence of V_e, which has been regarded as linear over the low concentration region. We assume that V_e can be expressed as a sum in the form[13]:

$$V_e = V_0 + K_d{}^{SE} V_i{}^{SE} + K_0{}^P V_i{}^P + K_0{}^S V_i{}^S \qquad (9)$$

where *SE, P* and *S* are respectively the contributions of steric exclusion, thermodynamic partition and sorption of solute on the gel. A decrease in the effective hydrodynamic volume of coils considered in this paper accounts for the changes of the coefficient K^{SE} with the concentration and leads to the linear dependence of V_e on c. Generally also the coefficients $K_d{}^P$ and $K_d{}^S$ are not constant and are concentration dependent and the adsorption and partition isotherms are not linear. For adsorption of polymers on a carrier the isotherms show such a course that the stationary phase is saturated at increasing concentration c in the mobile phase (Langmuir isotherm-mirror image of the isotherm in *Figure 8a*). For this type of isotherm, $K_d{}^S(c)/K_d{}^S(0) < 1$ and the relative contribution to the elution volume caused by adsorption of solute on a carrier decreases with increasing concentration. Similar behaviour can be expected also for the contribution to V_e caused by thermodynamic partition of solute between binary eluent in the stationary and mobile phase which can differ in their composition[13]. However, if the adsorption or partition isotherm has an anti-Langmuir form (as seen in *Figure 8a*), their contribution to the total V_e increases with c.

In general, when employing binary eluents and non-compressible gels, the finite concentration effect is given by a sum of all three concentration dependent distribution coefficients from equation (9). The resulting isotherm determining the separation process is given by superposition of all three isotherms; its shape is determined mainly by a contribution from steric exclusion excepting the region of very low concentrations (*Figure 9a*). The concentration dependence for the resulting isotherm as well as for its individual contributions are schematically shown in *Figure 9b*. It is seen that by using binary eluents, sorption and partition may cause deviations from the linear dependence of V_e on c in the very low concentration region upwards or downwards in the case of Langmuir or anti-Langmuir isotherm respectively. Experimental examination of the shape of V_e vs. c in a very low concentration region is relatively difficult in the usual g.p.c. experiment. In any case, however, the assumed deviation from the linearity of the concentration effect in the range of low concentrations can be neglected for correlation of the slopes of concentration dependences with the thermodynamic quality of eluent as seen from the course of concentration dependences in *Figures 1–4*.

REFERENCES

1. Cantow, M. R. J., Porter, R. S. and Johnson, J. F. *J. Polym. Sci. (B)* 1966, 4, 707
2 Lambert, A. *Polymer* 1969, **10**, 213

3 Berger, K. C. and Schulz, G. W. *Makromol. Chem.* 1970, **136**, 221

4 Berek, D., Bakoš, D., Šoltés, L. and Bleha, T. *J. Polym. Sci. (Polym. Lett. Edn)* 1974, **12**, 277

5 Boni, K. A., Sliemers, F. A. and Stickney, P. B. *J. Polym. Sci. (A-2)* 1968, **6**, 1567

6 Troth, H. G. *5th Int. GPC Seminar, London* 1968

7 Vilenchic, L. Z. and Belenky, B. G. *J. Chromatogr.* 1971, **56**, 13

8 Rudin, A. *J. Polym. Sci. (A-1)* 1971, **9**, 2587

9 Rudin, A. and Hoegy, H. W. *J. Polym. Sci. (A-1)* 1972, **10**, 217

10 Berek, D., Bakoš, D., Bleha, T. and Soltés, L. *Makromol. Chem.* 1975, **176**, 391

11 Kato, Y. and Hashimoto, T. *Kobunshi Kagaku* 1973, **30**, 107

12 Kato, Y. and Hashimoto, T. *J. Appl. Polym. Sci.* 1974, **18**, 1239

13 Bakoš, D., Bleha, T., Ozimá, A. and Berek, D. *J. Appl. Polym. Sci.* submitted

14 Kunst, E. D. *Dissertation Univ.* Groningen, (1950) quoted in 'Polymer Handbook' (Eds Brandrup and E. H. Immergut), Interscience, New York, 1966

15 Yamakawa, H. 'Modern Theory of Polymer Solutions', Harper and Row, New York, 1971

16 Yamamoto, A., Fujii, M., Tanaka, G. and Yamakawa, H. *Polym. J.* 1971, **2**, 799

17 Schulz , G. V., Baumann, H. and Darskus, R. *J. Phys. Chem.* 1966, **70**, 3647

18 Utracki, L. A. *J. Paint Technol.* 1972, **44**, 86

19 Rudin, A. and Wagner, R. A. *J. Appl. Polym. Sci.* 1976, **20**, 1483

20 Benoit, H., Grubisic, Z., Rempp, P., Decker, D. and Zilliox, J. G. *J. Chim. Phys.* 1966, **63**, 1507

21 Krigbaum, W. R. *J. Polym. Sci.* 1958, **28**, 213

22 Fixman, M. and Peterson, J. M. *J. Am. Chem. Soc.* 1964, **84**, 3524

23 Eizner, J. E. *Vysokomol. Soedin* 1961, **3**, 748

24 Simha, R. and Zakin, J. L. *J. Chem. Phys.* 1950, **33**, 1791

25 Winzor, D. J. in 'Physical Principles and Techniques of Protein Chemistry', Part A, (Ed S. J. Leach), Academic Press, New York, 1969

26 Gilbert, G. A. and Kellet, G. L. *J. Biol. Chem.* 1971, **246**, 6079

27 Baghurst, P. A., Nichol, L. W., Ogston, A. G. and Winzor, D. J. *Biochem. J.* 1975, **147**. 575

28 Nakano, S. and Goto, Y. *J. Appl. Polym. Sci.* 1975, **19**, 262