

# Contribution of Adsorption and Partition to the Separation Mechanism in Gel Chromatography on Inorganic Carriers

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## Synopsis

Gel permeation chromatography (GPC) calibration curves log hydrodynamic volume versus elution volumes were obtained from a series of single and mixed eluents for polystyrene on inorganic carriers. The observed calibration shifts were interpreted as a result of adsorption and partition effects on elution volume  $V_e$ . Secondary contributions to the separation mechanism can be qualitatively described by means of thermodynamic relations from liquid adsorption (LSC) and liquid partition (LLC) chromatography using the solubility parameters of the eluents. The universal calibration procedure based on the hydrodynamic volume of the coils is directly applicable only for systems in which adsorption and partition is approximately the same.

## INTRODUCTION

The steric exclusion of molecules from the pores of a carrier is considered in gel chromatography as a primary separation mechanism. The product of the limiting viscosity number and the molecular weight  $[\eta]M$  proportional to hydrodynamic volume of a macromolecule was proposed<sup>1</sup> as a universal parameter in GPC calibration. It should be valid for polymers of various types and structures as well as for various solvents.

However, a number of authors reported the dependence of universal calibration (UC) on the type of polymer, carrier, and eluent.<sup>2-8</sup> The observed shifts were most pronounced when mixed eluents were applied.<sup>2,7</sup> We compared chromatographic behavior of polystyrene (PS) in thermodynamically good and  $\theta$  eluents.<sup>2</sup> In the present paper we extend the study of solvent effects on UC to the intermediately good eluents for PS. We attempted to semiquantitatively interpret the shift of universal calibrations for binary eluents in comparison with single eluents on the basis of theoretical views on the difference of adsorption and partition between single and binary eluents.

## EXPERIMENTAL

Two Inox steel columns ( $3/8 \times 1050$  mm<sup>2</sup>) were packed with inorganic carriers Porasil D and Porasil E. A differential refractometer (Knauer 2025/50) was used as detector. The experiment was performed at a constant temperature of 25°C and a flow rate of 1 ml/min.<sup>2</sup> In addition to the eluents studied earlier, viz., benzene, chloroform, and the mixtures benzene-methanol (77.8:22.2) and chloroform-methanol (74.7:25.3),<sup>2</sup> binary eluents of composition benzene-

methanol (90:10), chloroform-methanol (90:10) were applied. All compositions of the mixture are given in volume ratios before mixing. Mixtures of 2-butanone (MEK) and *n*-heptane in the ratio 50:50, 68.4:31.6 and pure MEK were used as a model of the binary eluent containing a nonpolar component as a precipitant for PS. Elution volumes of PS standards (Pressure Chem. Co.) used in UC were obtained by linear extrapolation of concentration dependences to zero concentration of the injected sample.<sup>2,9</sup> The limiting viscosity numbers of PS were estimated from literature data<sup>10</sup> for *K* and *a* (Table I) and by using the relation  $[\eta] = KM^a$ . For mixtures MEK-*n*-heptane 68.4:31.6,  $[\eta]$  was determined viscometrically at 25°C by an Ubbelohde viscometer. The mixture MEK-*n*-heptane 50:50 represents a thermodynamically poorer eluent than a  $\theta$  solvent. The universal calibration in this case was constructed by using  $[\eta]$  data for the theta mixture (Table I).

## RESULTS AND DISCUSSION

The solute elution volume  $V_e^{SE}$  in GPC separation which is based on steric exclusion is expressed as

$$V_e^{SE} = V_0^{SE} + K^{SE}V_i^{SE} \quad (1)$$

where  $V_0^{SE}$  is the interparticle or "dead" volume,  $V_i^{SE}$  is the volume of the quasi-stationary phase of the solvent inside the gel, and  $K^{SE}$  is the distribution coefficient that describes the part of the inner volume which is accessible to the given solute. In the case of linear flexible macromolecules, the distribution coefficient can be interpreted as a change in the configuration entropy due to the polymer coil passing into the pores of the gel.<sup>11,12</sup>

Generally, the separation of the compounds based on different enthalpic effects is more frequent in chromatography. These effects arise from mutual interactions among carrier, mobile and stationary phases, and solute. Effects such as adsorption of the solute on the gel or partition of the solute between the mobile and stationary phases, etc., contribute to the value of  $V_e$  even in a special case of GPC:

TABLE I  
Parameters of Eluents

Eluent	Volume ratio before mixing	$K \times 10^{-5}$ (ref. 10)	<i>a</i> (ref. 10)	$\epsilon_{\text{Al}_2\text{O}_3}^0$ (ref. 15)	$\delta$ (ref. 15)
Benzene		9.2	0.74	0.32	9.2
Chloroform		7.16	0.76	0.40	9.1
Methyl ethyl ketone (MEK)		39.0	0.58	0.51	9.3
Chloroform-methanol	90/10	7.7	0.75	0.71	9.5
Chloroform-methanol	75/25 (theta)	73.0	0.5	0.87	10.1
Benzene-methanol	90/10	17.3	0.68	0.72	9.6
Benzene-methanol	77.8/22.2 (theta)	89.0	0.5	0.85	10.0
MEK- <i>n</i> -heptane	68.4/31.6	30.5 <sup>a</sup>	0.6 <sup>a</sup>	0.41	8.9
MEK- <i>n</i> -heptane	50/50	82.0 <sup>b</sup>	0.5 <sup>b</sup>	0.34	8.35

<sup>a</sup> Determined experimentally.

<sup>b</sup> Average value obtained from the  $\theta$  mixture data for PS.<sup>10</sup>

$$V_e = V_0 + K^{SE}V_i^{SE} + K^SV_i^S + K^PV_i^P + \dots \quad (2)$$

where  $K^S, K^P, \dots$ , represent the distribution coefficients of adsorption, partition, and all the other secondary mechanisms of the separation in GPC. Analogously, the relative elution volume  $V_e - V_0$  can be expressed as a sum of contributions of the individual mechanism,  $V_e^{SE}, V_e^S, V_e^P$ , etc.

Equation (2) can be formally written in the form of eq. (1) replacing the distribution coefficient  $K^{SE}$  by  $K'$ .  $K'$  is an effective measure of separation which also involves other secondary mechanisms besides the predominant contribution of the steric exclusion. Expression of the universal calibration (UC) by the effective hydrodynamic volume of polymers in solution proportional to the value  $M[\eta]$  is based on a similar principle. When comparing the elution behavior of polymers in a series of solvents in which the secondary effects (contributions  $V_e^S, V_e^P, \dots$ ) are equal, the effective distribution coefficient of GPC separation,  $K'$ , is determined only by the steric exclusion contribution  $V_e^{SE}$  and thus by the sizes of the separated molecules. It can be expected that for chemically similar eluents, the differences in secondary effects will be small and the UC will coincide within the experimental error. A different situation should arise when comparing eluents, where polarity and affinity to the gel and solute differ markedly. Here, however, the polarity difference of single eluents is limited by the solubility of polymer solutes.

Figure 1 shows the noncoincidence of UC curves for PS in single and in mixed eluents. The UC curves are shifted to the lower elution volumes when the eluent contains methanol, while the shift to higher  $V_e$  is observed for *n*-heptane-containing solvents. The shifts increase with increasing amounts of precipitants and, in the case of methanol-containing eluents, with decreasing molecular weights of solute. This disagreement of the UC does not disappear even with a change in viscosity constant  $\phi$  of the solvent e.g., according to Ptitsyn and Eizner,<sup>13</sup>

$$\phi(\epsilon) = 1 - 2.63\epsilon + 2.86\epsilon^2 \quad (3)$$

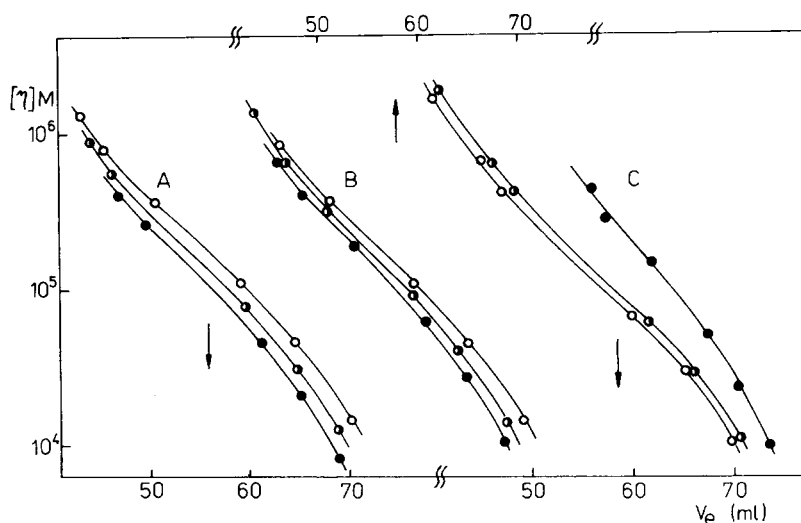


Fig. 1. Universal calibration curves for polystyrene in eluents. (○) chloroform; (●) chloroform-methanol (90/10); (◐) chloroform-methanol (75/25). (○) benzene; (●) benzene-methanol (90/10); (◐) benzene-methanol (77.8/22.2). (○) MEK, (◐) MEK-*n*-heptane (68.4/31.6); (●) MEK-*n*-heptane (50/50). Volume ratios before mixing.

where  $\epsilon = (2a - 1)/3$ , and  $\log [\eta]M/\phi^{(\epsilon)}$  versus  $V_e$  (Fig. 2) are plotted.

The problems of UC for PS on an inorganic carrier is not limited to  $\theta$  solvents only,<sup>2</sup> although the displacement of UC increases as the mixed eluent becomes thermodynamically poorer.

We tried to account qualitatively for the deviations observed in universal calibrations with mixed eluents.<sup>2</sup> It was shown that the differences in the solute-gel interaction are influenced by the polarity of the mixed eluent components. In this study we evaluate more quantitatively the role of adsorption and partition in GPC with mixed eluent, though the mutual separation of their contributions is rather difficult.

### EFFECTS OF ADSORPTION

Let us compare the situation for benzene and its mixtures with methanol. Porasil used as a GPC carrier contains numerous adsorption sites, mainly in the pores, since the contribution of the external surface of particles to the overall active surface is negligible. The adsorption sites are solvated by benzene molecules which act as an eluent. Since PS is chemically similar to benzene, the solvent-gel and solute-gel interactions per monomer unit should be comparable. On passing the PS-containing zone through the column, a dynamic competitive equilibrium is established between the molecules of benzene and PS on the adsorption sites of the carrier. Consequently, the  $V_e$  of PS will be larger than  $V_e^{SE}$  (corresponding to the "pure" steric exclusion) owing to small adsorption retardation. If, however, a mixture of benzene and methanol is used as the eluent, methanol, as a more polar component, preferentially occupies adsorption sites on the carrier as shown by static measurements<sup>2</sup> and supported by literature data.<sup>14</sup> The passing zone with PS molecules displaces practically no methanol from adsorption sites. In this case there should be substantially fewer adsorption sites solvated by benzene. The retardation of PS in the column should be, as a result of adsorption, smaller in methanol mixtures than in pure benzene (Figs.

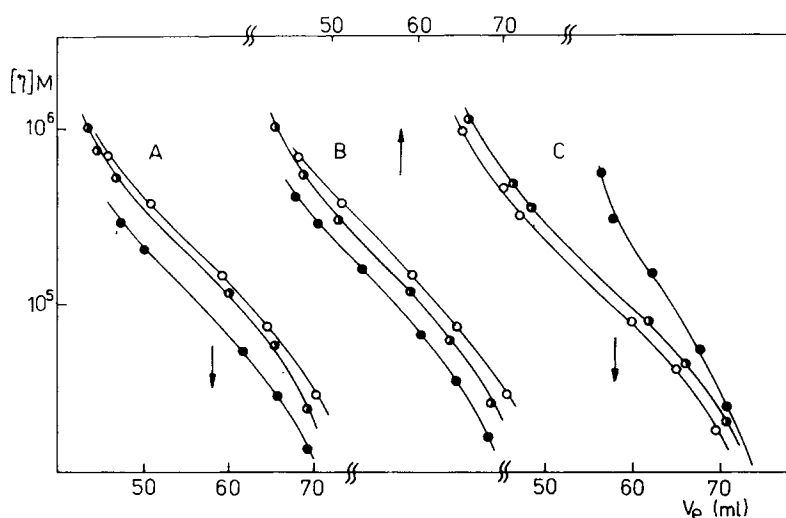


Fig. 2. Universal calibration curves with considered change of constant  $\phi$  according to Ptitsyn and Eizner.<sup>13</sup> Symbols same as in Fig. 1.

1 and 2). An analogous explanation can be used for chloroform and its mixtures with methanol. In pure MEK it is assumed that similarly as in benzene and chloroform adsorption contributes partly to the  $V_e$  of injected PS samples, as a consequence of competition between molecules of eluent and PS for adsorption sites. Addition of the nonpolar component *n*-heptane to MEK affects this equilibrium, but MEK (as a more polar component) still preferentially solvates the majority of adsorption sites on the gel. *n*-Heptane, which occupied some adsorption sites, is displaced by the passing zone of PS, since the latter has greater affinity to the gel. This leads to retardation of PS in mixtures of MEK with *n*-heptane (Figs. 1 and 2). The corresponding  $V_e$  decreases with the increasing polarity of the eluent. The UC for binary eluents depending on their polarity can be displaced to higher or lower  $V_e$  in comparison with UC for single eluents.

The effect of solvent upon elution behavior in adsorption liquid chromatography was studied in detail by Snyder.<sup>15</sup> For the relative elution volume in the adsorption chromatography,  $V_e^S - V_0$ , the relation is

$$\log (V_e^S - V_0) = \log V_a + \alpha(S^0 - \epsilon^0 A_i) \quad (4)$$

and for the ratio in two solvents, using the same carrier ( $V_a$  and  $S^0$  are constants)

$$\log \frac{(V_e^S - V_0)_1}{(V_e^S - V_0)_2} = \alpha A_i (\epsilon_2^0 - \epsilon_1^0) \quad (5)$$

$V_a$  is the adsorbent surface volume,  $\alpha$  is the parameter characterizing the activity of adsorbent,  $S^0$  is the dimensionless adsorption energy of solute from pentane to adsorbent with the standard activity ( $\alpha = 1$ ),  $A_i$  is the effective molecular surface of solute, and  $\epsilon^0$  is the "eluent strength" determined by the magnitude of the adsorption energy per unit of the adsorbent surface.

For comparison of shifts in UC in Figures 1 and 2, it is necessary to express the difference  $\Delta V_e$  instead of the ratio of elution volumes in two eluents. For the difference of elution volumes  $\Delta V_e^S$  from the adsorption effects referred to 1 g of a carrier it follows from eq. 4 that

$$(V_e^S)_1 - (V_e^S)_2 = \Delta V_e^S = V_a \times 10^{\alpha S^0} [10^{-\alpha A_i \epsilon_1^0} - 10^{-\alpha A_i \epsilon_2^0}] \quad (6)$$

For a polymer of certain molecular weight, solute, and given carrier,  $S^0$ ,  $V_a$ ,  $\alpha$ , and  $A_i$  are constants which are not affected by the change of eluent. The variation of quality of solvent can be accompanied by expansion or contraction of the polymer coils, which might affect the value  $A_i$ ; this change, however, can be neglected.

The difference in elution volumes  $\Delta V_e$  for UC, e.g., of the methanol-containing systems, may be assigned to different values of adsorption of the solute on the carrier expressed by  $\epsilon^0$  parameters, eq. (6). Explanation of the molecular weight dependence of the shift,  $\Delta V_e$ , is more complicated. The universal calibrations are not mutually shifted by a constant increment in the whole range of molecular weights, where the separation is effective (Figs. 1 and 2). With increasing degree of polymerization mainly the effective area of the coil for adsorption,  $A_i$  will increase. Simultaneously, smaller pores will become inaccessible for the coils, and the  $V_a$  value thus decreases. These effects might account for the dependence  $\Delta V_e = f(M)$  observed.

Snyder<sup>15</sup> gives the values of  $V_a$ ,  $\alpha$ ,  $A_i$ , and  $\epsilon^0$  for the most frequently used solvents and carriers in adsorption chromatography of low molecular weight compounds. However, direct application of these data to the systems with the carrier Porasil and PS as the solute is not possible, and it would require independent determination of parameters in eq. (6). The situation can be partially simplified by substituting the solubility parameter  $\delta$  for  $\epsilon^0$ . The values of  $\delta$  are well known for common eluents. The linear correlation between  $\epsilon^0$  and  $\delta$  was found by Keller et al.<sup>16,17</sup> It follows from eq. (6) that the shift  $\Delta V_e^S$  of UC, due to difference in solute adsorption for a couple of eluents, depends on differences in their  $\delta$  parameters. It seems, however, that in the case of mixed eluents the correlation between  $\epsilon^0$  and  $\delta$  does not involve preferential adsorption of one of the components of eluents on the gel.<sup>17</sup>

In eq. (6) if powers will be expanded as a series up to the third term and the constant term will be replaced by  $k_1$  and  $k_2$ , we obtain

$$\Delta V_e^S = -k_1(\epsilon_1^0 - \epsilon_2^0) + k_2(\epsilon_1^2 - \epsilon_2^2) \quad (6a)$$

Alternatively, if the assumed proportionality between  $\epsilon^0$  and  $\delta$  (17) is considered, after rearrangement we obtain

$$\Delta V_e^S = -k_1'(\delta_1 - \delta_2) + k_2'(\delta_1^2 - \delta_2^2) \quad (6b)$$

If we assumed that the displacement of the universal calibration  $\Delta V_e$  (Figs. 1 and 2) arises from solute-gel adsorption phenomena, both eqs. (6a) and (6b) can be employed in the quantitative interpretation of this displacement. The constants  $k_1$ ,  $k_1'$ ,  $k_2$ , and  $k_2'$  are considered as empirical parameters and benzene is used as reference eluent 1. The  $\delta$  and  $\epsilon^0$  values for all eluents of this and the previous paper<sup>2</sup> summarized in Table I are taken from Snyder<sup>15</sup> or calculated for mixed eluents according to his method. For solvent strength  $\epsilon^0$  on Porasil we assumed  $\epsilon_{\text{Porasil}}^0 = \epsilon_{\text{silica}}^0 = 0.77 \epsilon_{\text{alumina}}^0$ . The volume fraction additivity criterion of individual component was used for calculation of  $\delta$  parameter of mixed eluents.

Figures 3 and 4 show the correlation of the shift of the universal calibration  $\Delta V_e$  (from Fig. 2 determined approximately in the middle of the separation region in value  $M[\eta] = 10^5$ ) with  $\epsilon_2^0$  and  $\delta_2$ , respectively.  $\Delta V_e$  values for mixtures with methanol are positive and increase with increasing content of methanol and thus with increasing  $\epsilon_2^0$  and  $\delta_2$  for mixed eluents.

The comparison of UC of three single eluents reveals a significant shift of MEK data to lower  $V_e$  in comparison with benzene and chloroform. The plots for MEK and its mixtures with *n*-heptane (Figs. 3 and 4) indicate a different course in comparison with methanol-containing mixtures. In the cases of *n*-heptane-containing mixtures, partition becomes more marked.

Complete retardation of PS was observed<sup>2</sup> in  $\text{CCl}_4$  and in a mixture of benzene-*n*-heptane (1:1), i.e., in this case  $\Delta V_e \rightarrow -\infty$ . It seems that in the region of nonpolar eluents there exists a critical value for  $\epsilon^0$  and  $\delta$  below which a column total PS adsorption occurs. At slightly higher  $\epsilon^0$  and  $\delta$  the comparable magnitude of both the steric exclusion and the adsorption contribution to  $V_e$  is to be expected.

The preceding treatment is based on assumptions valid in LSC of low molecular weight compounds where the dominant separation factor is the enthalpic component of the distribution coefficient  $K^S$ . One cannot expect eq. (6) to ex-

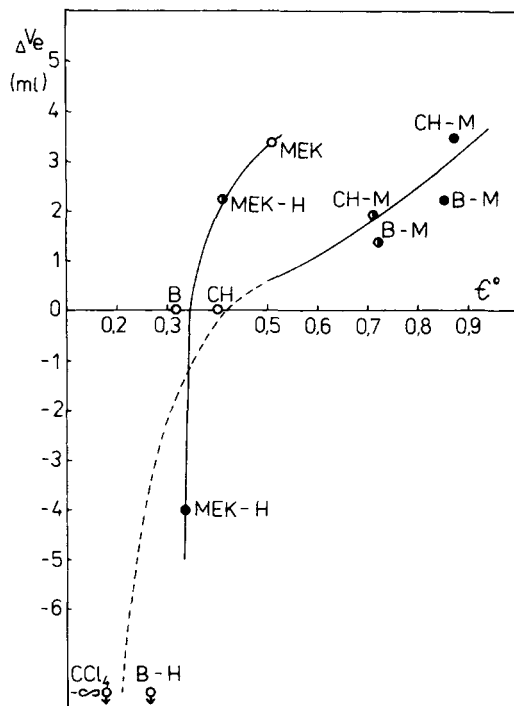


Fig. 3. Correlation of shift in universal calibration  $\Delta V_e$  with parameter  $\epsilon_2^0$  (relative to benzene as reference value  $\epsilon_1^0$ ).

actly describe the adsorption phenomena in the case of GPC of polymers on inorganic carriers where the entropic contributions to  $K^S$  cannot be neglected. This relation represents an attempt to elaborate more quantitatively the correlation between the shifts of universal calibrations in Figures 1 and 2 and the parameter  $\epsilon^0$ .<sup>2</sup>

### EFFECTS OF PARTITION

We dealt with sorption phenomena by comparing UC of the binary mixtures and single eluents. Due to preferential solvation of the gel by one of the components of the mixed eluent, a difference in the thermodynamic quality of the mobile and stationary phases and, consequently, additional partition of the solute may occur. In the case of methanol-containing mixtures, methanol is adsorbed on the gel, i.e., the quasi-stationary phase is enriched by the precipitant for PS so that partition as a secondary separation mechanism in GPC does not play a significant role. A different situation arises for mixtures containing *n*-heptane, where MEK (thermodynamically better solvent for PS) is preferentially adsorbed on the gel. The mobile phase then becomes thermodynamically poorer than the quasistationary phase adsorbed on the gel and the thermodynamic partition of the solute between both phases may occur. Although this effect is probably much more significant of organic gels,<sup>22</sup> it is possible that it contributes substantially to the shift of universal calibrations in Figures 1 and 2 for the systems with *n*-heptane.

Let us assume that the shift of UC in mixtures with MEK can be assigned to

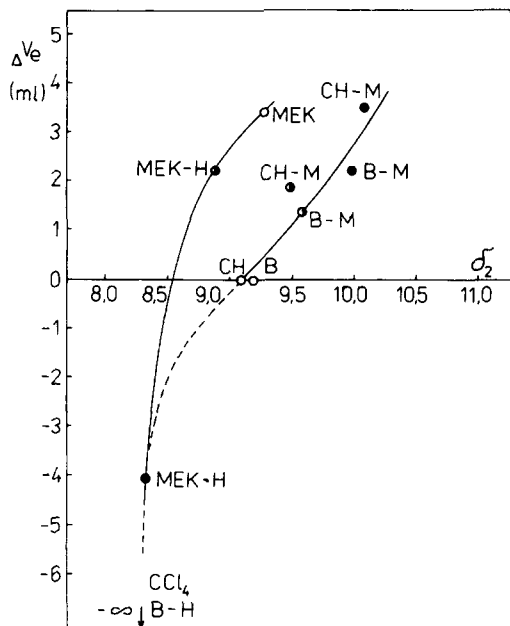


Fig. 4. Correlation of shift in universal calibration  $\Delta V_e$  with parameter  $\delta_2$  (relative to benzene as reference value  $\delta_1$ ).

this partition effect. For relative elution volume in LLC chromatography per 1 g of carrier,<sup>18</sup>

$$V_e^P - V_0 = \frac{\gamma^{m,\infty} M_m}{\gamma^{s,\infty} M_s \rho_m} \quad (7)$$

where  $M_m$  and  $M_s$  are the molecular weights of the liquid forming the mobile and stationary phases, respectively; and  $\gamma^{m,\infty}$  and  $\gamma^{s,\infty}$  are the solute activity coefficients at infinite dilution. Equation (7) holds for sufficiently diluted solutions, where the mutual interactions of solute molecules are negligible and only the solvent-solute interaction is decisive.

Let us assume that the activity coefficient of polymer solute (PS) can be characterized by the Flory-Huggins equation<sup>19</sup>

$$\ln \gamma^\infty = \ln \frac{1}{r} + \left(1 - \frac{1}{r}\right) + \chi \quad (8)$$

where  $r = v_1/v_2$  is the ratio of molar volumes of the solvent and polymer and  $\chi$  is the interaction parameter. Activity coefficients of PS for the stationary phase enriched by MEK and the mobile phase will differ mainly in the value of  $\chi$ . Here, the difference in molar volumes of MEK and *n*-heptane is neglected and  $r$  is considered constant for both phases. On substituting Eq. (8) into (7) we obtain

$$\Delta V_e^P = \frac{M_m}{M_s \rho_m} \exp(\chi^{m,\infty} - \chi^{s,\infty}) \quad (9)$$

The contribution to  $V_e$  caused by partition of the solute into the stationary phase is, according to eq. (9), a function of the Flory-Huggins interaction parameter



$\chi$ . The higher its value the less favorable is the formation of the pair contact between the types of molecules described.

Equation (7), derived originally in LLC chromatography of two immiscible liquid phases, was modified for the case of the chemically bonded nonpolar stationary phase.<sup>20</sup> Its direct application to estimate the shift  $\Delta V_e^P$  owing to partition in GPC on inorganic carriers is not straightforward. In the first place, the composition of the MEK-enriched stationary phase as well as the dependence of the effective interaction parameter  $\chi$  (for ternary system solute plus binary eluent) on the composition of binary eluent, i.e., on the binary parameters  $\chi_{12}$ ,  $\chi_{13}$ , and  $\chi_{23}$ , should be known.

Equation (9) can be simplified in a first approximation using the solubility parameter concept. If the validity of the theory of regular solutions modified by the Flory-Huggins expression of ideal mixing entropy for the components in solution differing in their sizes is assumed, the  $\chi$  parameter may be expressed<sup>21</sup> by the solubility parameters of the solvent  $\delta$  and polymer  $\delta_p$

$$\chi = \frac{v_1}{RT} (\delta - \delta_p)^2 \quad (10)$$

It is known that the better a solvent is for a given polymer, the closer is its  $\delta$  parameter to  $\delta_p$  of the polymer. Substituting eq. (10) into (9), it follows that the partition of solute into the stationary phase occurs if the solubility parameter of stationary phase  $\delta_s$  is closer to  $\delta_p$  than the solubility parameter of the mobile phase  $\delta_m$ .

For the case of mixtures of MEK and *n*-heptane, it is assumed that their  $\delta$  parameter is determined additively from  $\delta$  parameters of both components.<sup>21</sup> Since the stationary phase is enriched by MEK (a thermodynamically good solvent for PS), it holds that  $(\delta_s - \delta_p)^2 < (\delta_m - \delta_p)^2$  or, substituting into eq. (10),  $\chi^{m,\infty} > \chi^{s,\infty}$ . According to eq. (9), it ensues that partition may contribute to the difference in universal calibrations for PS in MEK and its mixtures with *n*-heptane. The more exact evaluation of  $\Delta V_e^P$  in this case would require at least the crude estimation of composition of both the stationary and the mobile phases.

The semiquantitative character of correlations [eqs. (9) and (10)] has to be underlined with regard to the number of assumptions for validity of the real GPC system. Moreover, the separate interpretation of adsorption and partition can lead to the fact that some contributions to the shift of the universal calibrations will be included twice. Both of these effects lead to retardation of the solute and increase in elution volumes.

## UNIVERSAL CALIBRATIONS AND SECONDARY SEPARATION EFFECTS

The concept of universal calibrations ( $\log [\eta]M$  vs  $V_e$ ) is based on the comparison of the effective sizes of macromolecules in various solvents. Figures 1 and 2 demonstrate the significant role of secondary effects on inorganic carriers in GPC. Therefore, these effects ought to be considered in a "modified" universal calibration which would encompass the effect of both factors, namely, the size of solute and molecular interactions in the chromatographic system. It is rather difficult to find a simple and universal expression for molecular interaction

effects. The preceding considerations show that the  $\delta$  parameter might serve as a crude estimate of secondary separation contributions to  $V_e$ .

The universal calibration, based on the hydrodynamic volume, can still be used, but only if  $\log M[\eta]$  versus  $(V_e)_{\text{corr}}$  is plotted.  $(V_e)_{\text{corr}}$  is the elution volume of solute corrected for the difference in molecular interactions (adsorption, partition, etc.) in compared eluents, i.e.,

$$(V_e)_{\text{corr}} = V_e + \Delta V_e \quad (11)$$

Even though  $(V_e)_{\text{corr}}$  represents the effective hydrodynamic volume of molecules, it should be differentiated from steric exclusion contribution to elution volume  $V_e^{SE}$  in eq. (1). The  $V_e^{SE}$  contribution in  $(V_e)_{\text{corr}}$  also includes that part of the molecular interaction which is common to both compared solvents, and only their difference (or excess for one eluent) is separated into the  $\Delta V_e$  term.

The displacement of the universal calibration (or the  $\Delta V_e$  term) depends on the molecular weight (Figs. 1 and 2). The independent experimental determination of the  $\Delta V_e$  term is difficult, but eqs. (6) and/or (9) can be used for its a priori estimation.

The validity of the proposed correction of the  $\log M[\eta]$  versus  $V_e$  calibration, due to difference in molecular interactions, is also supported by results for normal calibration  $\log M$  versus  $V_e$ , e.g., for system with benzene and its mixture with methanol. The mutual crossing of normal calibration curves and their reverse order in the region of low molecular weights are shown in Figure 5(a). After correction of the normal calibrations of the benzene and benzene-methanol (90:10) mixture with respect to the  $\theta$  mixture by the  $\Delta V_e$  value from the UC in Figure 1 we obtain Figure 5(b). This plot is now compatible with the idea that the poorer the thermodynamic quality of the eluent, the more the calibration curve is shifted toward higher  $V_e$  over the entire range of molecular weights.

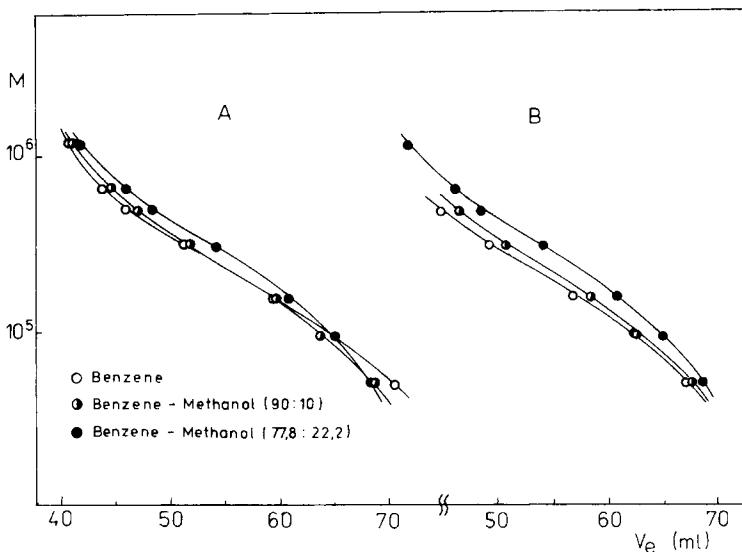


Fig. 5. Uncorrected (a) and corrected (b) calibrations for benzene and its mixtures with methanol. For specification of experimental points see Fig. 1.

The proposed modification of the universal calibration in the form  $\log M[\eta]$  versus  $(V_e)_{\text{corr}}$  agrees with the literature since the universal calibration  $\log M[\eta]$  versus  $V_e$  for one polymer was compared most frequently for the solvents of similar thermodynamic quality and polarity. For example, in this case  $\Delta V_e \approx 0$  and  $(V_e)_{\text{corr}} = V_e$  within experimental error. The use of binary eluents broadens the region of solvent polarity and a larger displacement of universal calibrations was observed. The displacement of UC for MEK in comparison with benzene and chloroform (Figs. 3 and 4) suggests, however, that this effect also occurs in single eluents of different polarity.

Recently, similar interpretations of displacement of universal calibrations of PS in good theta solvents on crosslinked PS gels based on solute-gel interactions were proposed by Dawkins and Hemming.<sup>22</sup> They introduced the distribution coefficient  $K_D$  as a measure of solute-gel interaction which is unity in good solvent media separating solely by steric exclusion. Its value for poor eluents should be fitted from experimentally formed universal calibrations in a similar method as parameter  $\Delta V_e$ . The advantage of our procedure consists in unified treatment of solvent effects on solute-gel interactions in GPC with similar effects in LSC and LLC and in a priori prediction of displacement from physicochemical parameters of eluents such as  $\epsilon^0$ ,  $\delta$ , etc.

Evaluation of adsorption and partition effects in GPC is of importance from the practical point of view. The secondary effects, if neglected, may bring about a considerable error in determining the molecular weight of the polymer as well as deformation of the molecular weight distribution. The magnitude of these effects is also a function of the molecular weight of the polymer (Figs. 1, 2, and 5). These secondary separation effects may also occur in systems using single eluents which are, in principle quasi-binary systems. The typical examples are THF and other hydroscopic solvents containing water or chloroform usually stabilized with ethanol, etc. Thus, the necessity to work with the highly pure solvents or at least with solvents with constant compositions is evident.

The semiquantitative description of the corrections of UC employed in the present paper for the experimental results with inorganic carriers might in principle be extended to organic gels. But, in contrast to inorganic gels, partition in binary eluents will play a much more significant role. The contribution of partition expressed in eq. (9) by the effective interaction parameters  $\chi$  in the stationary and the mobile phases can be correlated with the coefficient of preferential solvation of the polymer network  $\lambda$  in the case of organic gels.<sup>23</sup>

We have stressed the importance of the secondary separation mechanism in comparing the universal calibration based on the hydrodynamic volume of one polymer in different eluents. This consideration should also hold for the comparison of universal calibrations of chemically different polymers in the same eluent.

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