Thermodynamic and hydrodynamic properties of polymer — tetrahydrofuran — water systems: 2. Gel chromatography of polystyrene*

T. Spychaj† **and D. Berek**‡
Polymer Institute of the Slovak Academy of Sciences, 809 34 Bratislava, Czechoslovakia

(Received 10 November 1978, revised 30 January 1979)

Chromatogram shapes, concentration effects and calibration curves in g.p.c. were studied for dry tetrahydrofuran and its mixtures with water up to 8.9 vol. %. Polystyrene reference materials were separated with silica-based column packings. The results can be generalized fairly well and lead to the conclusion that the gel chromatographic data are considerably influenced by humidity present in the eluent. Ghost-peaks appeared in the domain of high elution volumes (V_e) using mixed eluents. Simultaneously, the solute peaks changed their widths and the slope of the plot of V_e *versus* injected polymer concentration changed with water content. However, most important were the shifts in the polymer elution volumes that were caused by the complex of interactions in the system gel–mixed eluent–solute and depended on the amount of water present in eluent. Thus, the dry eluent is an inevitable condition for obtaining g.p.c. results of high precision and reproducibility. The use of a guard column filled with a sorbent strongly trapping water is proposed in g.p.c. with hygroscopic eluents. Similar precautions are to be considered for any admixture in the eluent, especially if its content cannot be kept constant and if its polarity differs substantially from the polarity of the eluent.

INTRODUCTION

High dissolving power and low refractive index make tetrahydrofuran (THF) one of the most popular eluents in gel chromatography (g.p.c.). However, the hygroscopicity of this solvent may bring about several problems in its application. In the first part of this series[1] THF has been shown to absorb moisture from the air if stored in insufficiently closed vessels such as eluent containers in many commercial g.p.c. apparati.

The aim of the present study was to demonstrate the effects of water admixtures in THF eluent on the g.p.c. separation of polystyrene on SiO_2 based gels.

EXPERIMENTAL

Eluents

Dry tetrahydrofuran and mixtures of THF and water containing 2.0, 2.9, 4.5, 7.7, 8.2 and 8.9 vol % of water (volumes before mixing) were used. The purification and drying of the THF (VEB–Jenapharm, Laborchemie Apolda, GDR anal. grade) and the preparation of the mixtures of THF and water have already been described[1]. Mixed eluents except for azeotropes were not degassed in order to prevent gradient formation causing an unstable g.p.c. baseline.

Polystyrene

Narrow PS standards (Pressure Chemical Co., Pittsburgh, USA) were measured in the range of molecular masses $M = 4 \times 10^3 – 2 \times 10^6$. The narrow sample of PS ($M_{g.p.c.} = 1.2 \times 10^6$) was obtained from Centre de Recherches sur les Macromolécules CNRS, Strasbourg, France.

Apparatus

Two g.p.c. assemblies built up at our Institute were used. In both cases the detector was a differential refractometer Knauer, Model 2025/50 (Knauer KG, West Berlin). The glass siphon (about 4.50 ml) was calibrated for each set of experiments by weighing the effluent. System I contained two jacketted straight stainless columns 1220 × 8 mm filled with porous glass CPG–10 (Electro Nucleonics, Fairfield, USA) with nominal pore sizes 72 and 36.8 μm, respectively, and with 37–74 μm (200–400 mesh) particle size. The columns were thermostatted by a water thermostat to 25° ± 0.2°C. The elution rate was 0.7 cm^3/min and injected volumes were 1.43 cm^3. System II contained three columns (1220 × 8 mm) packed with CPG–10–1422, Porasil 500 and Porasil 150 (Rhône Progil, Vitry-sur-Seine, France). The mean pore size of the gels was respectively 142.2, 50 and 15 nm. The elution rate was 0.7 cm^3/min and injected volume 2.0 cm^3.

RESULTS AND DISCUSSION

Sorption measurements in the system gel–THF–water

Preliminary sorption measurements in the system CPG–10–368 (mean pore size 36.8 nm)–THF–water at 25°C

---

*Partly presented at the 5th International Symposium 'Progress and Application of Chromatography in the Chemical Industry', Bratislava, April 1977.

†Present address: Institute of Chemical Technology, Technical University of Szczecin, 70 322 Szczecin, Poland.

‡To whom correspondence should be addressed

0032–3861/79/091108–07$02.00

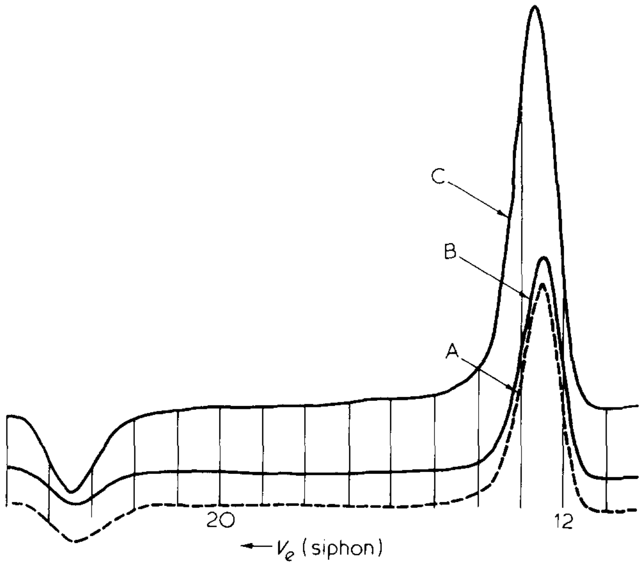

Figure 1 Typical chromatograms obtained in the systems PS–THF–water for PS $M = 670\,000$ in eluents containing 7.7 (——) and 8.2 (– –) vol. % of water. PS, $M = 6.7 \times 10^5$; ——, THF–water 7.7%; – – – THF–water 8.2%. A, $C_i = 1$ mg/ml; B, $C_i = 1$ mg/ml; C, $C_i = 4$ mg/ml

were performed in the static arrangement. Dry gel (1 g) was weighed into glass vials and poured into 9.3 cm^3 of the mixed solvent. The concentration of supernatant was measured by a differential refractometer Brice–Phoenix (Model BP–2000 V, Phoenix Precision Instr. Co., Philadelphia, USA) after 4 h of equilibration. An 'S' shaped sorption isotherm was found. As expected[2,3], water was preferentially sorbed in the gel from mixtures containing less than about 30 wt % of water. The maximum amount of adsorbed water was about 11 mg/gram of gel at about 16 wt % of water in the mixture. This corresponds to about 50% surface coverage.

Shape of chromatograms

Three typical chromatograms obtained with mixed eluents are shown in *Figure 1*. 'Positive' peaks of polymers are followed by smaller peaks of the opposite, i.e. 'negative', sign at high elution volumes. The latter peaks appeared in mixed eluents regardless of whether the eluent was degassed or not. Their size depended on the injected polymer concentration and on the eluent composition. Their elution volume was equal to that of water independently injected into columns. Evidently, the peaks mentioned were caused mainly by local changes in the eluent composition and we shall call them 'vacant peaks'[4]. The appearance of the vacant peak was shown[5] to be a general phenomenon in the liquid chromatography with mixed eluents. Local changes in the eluent composition may be caused by the following effects: preferential solvation of solute molecules; displacement effects; preferential evaporation effects; and absorption of moisture. The above effects with respect to the PS–THF–water system can briefly be discussed.

Preferential solvation of solute molecules. Polystyrene molecules are expected to be preferentially solvated by THF. This means that the bulk ('dialysed') solvent outside the solvation shells of the macromolecules is enriched by water. The zone of solvated macromolecules is separated from the zone of bulk solvent in the chromatographic column and a vacant peak appears on the chromatogram. Since water has a lower refractive index than THF, the peak of the bulk solvent is of 'negative sign'.

Displacement effects. The matrix of the gel is usually preferentially solvated by one of the mixed eluent components. The excess molecules may be displaced from the surface of the gel by solute molecules producing a displacement vacant peak[4,6]. We have already shown that the porous glass preferentially sorbed water from the mixtures of THF and water. In this case the displacement vacant peaks, if any, would correspond to the local excess of water and again have negative sign.

Preferential evaporation effects. Preferential evaporation of one component of the mixed solvent can take place while dissolving the sample and other manipulations with the injected solution. As a result, the peak of preferential evaporation is formed corresponding again to a local excess of water in the systems studied, since THF has a higher vapour pressure than water.

Absorption of moisture. The solutions prepared can absorb moisture from air and some additional water may also be added together with the polymer sample. Such effects can also be considered in this connection since water is a component of the mixed eluent in our case. Evidently, the absorption of moisture by sample solution will again produce a negative vacant peak.

The above results can be generalized and the appearance of a vacant peak is to be expected in any humid eluent. However, in the case of polar solutes preferentially solvated by water or in the case of a less polar gel e.g. polystyrene gel, the vacant peaks may have a positive or even changing sign dependent on which effect prevails. While the vacant peaks may complicate the interpretation of chromatograms in the region of low molecular masses of solutes, the preferential solvation of polymeric solute can also influence the shape of its peak[5]: the nonspecific detector-like differential refractometer responds to the solvated macromolecules. The extent of preferential solvation may depend on molecular mass of polymer[7] and on its concentration[8]. In such cases the response of the detector will be no longer linearly-dependent on actual polymer concentration and independent of its molecular mass.

The shape of solute peaks is, however, affected also by a number of other factors, such as eluent viscosity, concentration effects, etc., and is reflected in the resolution R_s of the given chromatographic system defined by relation[9]

$$R_s = \frac{2(V_{e_1} - V_{e_2})}{(w_2 + w_1)(\log \bar{M}_{w_1} - \log \bar{M}_{w_2})} \tag{1}$$

where V_{e_1} and V_{e_2} are the elution volumes of both polymers used for testing the column resolution, w_1 and w_2 are the corresponding peak widths at the baseline and $\bar{M}_{w_1}$ and $\bar{M}_{w_2}$ are the weight-average molecular masses of polymer reference materials.

The dependence of R_s on the concentration of injected polymer C_i for PS with $M = 4.98 \times 10^5$ and 2.33×10^5 is shown in *Figure 2* for four eluents studied. Evidently, the resolution of the given column system is a function of the water content in THF. The highest R_s values at low C_i, i.e. the best resolution, is for dry THF; however, the values for R_s decrease rapidly with increasing C_i in this eluent. The poorer the thermodynamic quality of the eluent for polystyrene, the smaller is the drop of R_s with C_i. This leads to the conclusion that the thermodynamically poor solvents for separated polymers are preferable eluents for preparative g.p.c. with high loads.

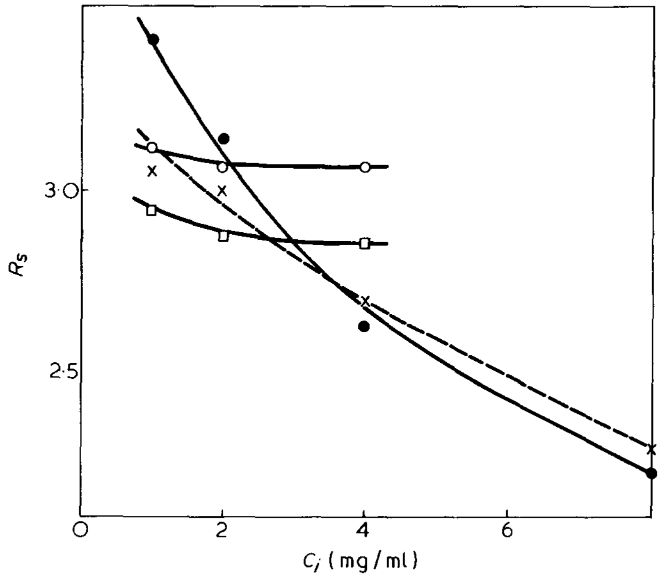

Figure 2 Dependence of resolution factor R_s on polymer concentration C_i for THF (●) and binary eluents THF–water containing 4.5 (X); 8.2 (□); and 8.9 (O) vol % of water. PS, 4.98 x 10^5; 2.33 x 10^5

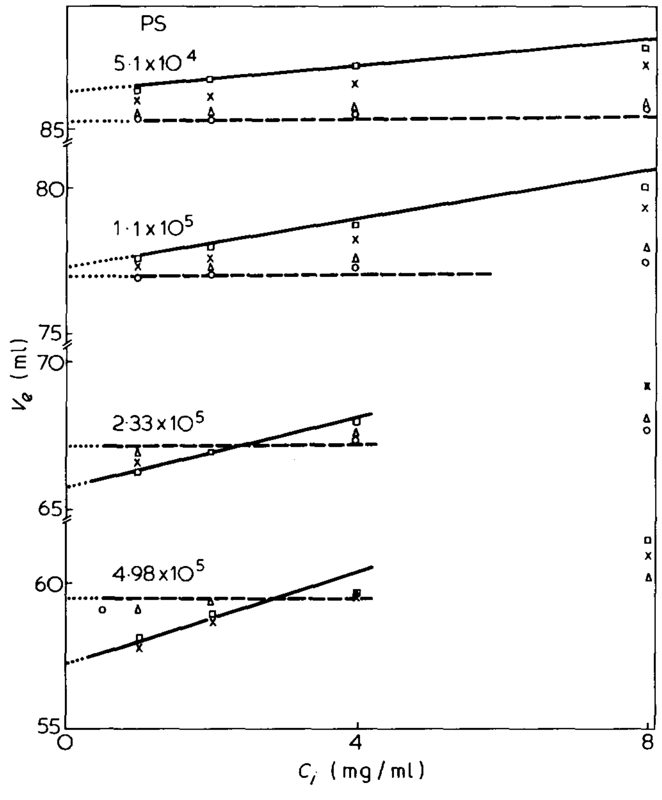

Figure 3 Concentration dependences of elution volumes of polystyrenes in THF (——) and binary eluents THF–water containing 2.0 (□); 4.5 (X); 7.7 (△); 8.2 (O); and 8.9 (– –) vol % of water

Concentration effects

Figure 3 shows the dependence of elution volume (V_e) on injected concentration (C_i) for four PS standards. For the sake of clarity, experimental points except for THF and the mixture containing 8.9% of water were omitted. It can be seen that V_e changes linearly with C_i in the concentration range between 0.5 and 4.0 mg/cm^3 and for the given polymer the slopes k of the corresponding straight lines:

(i) have their maximum values $k = k_{max}$ in pure THF and decrease with increasing amount of water in the eluent, i.e. with the decreasing thermodynamic quality of the mixed solvent for polystyrene molecules. This is in qualitative agreement with the results obtained with other (mixed) eluents[10,11];

(ii) are greater than zero in the eluents containing both 7.7 and 8.2% of water which are according to our turbidimetry and viscometry data inter- and intramolecular[12] theta mixtures for PS at 25°C [1]. However, $k \cong 0$ is observed in the mixture of THF and water 91.1/8.9 v/v which is a 'poorer' solvent compared with the theta solvent. Values for k slightly higher than zero were observed in some other theta systems[10] as well.

The straight lines V_e–C for different M obtained with particular mixed eluents seem to intercept at certain C_i. The loci of these intercepts change with polymer molecular mass. A similar phenomenon was observed in other systems[10,11,13]; however, any speculations in this respect need further experimental data.

The plot of k *versus* polymer molecular mass is given in *Figure 4* for the studied eluents. Clearly, k increases with M in the range of molecular masses where the columns efficiently separate. The maximum on the curve k–M coincides approximately with the upper limit of the 'linear' part of the calibration curve $\log M$–V_e (see curves 1 and 2 in *Figure 7*). An independent set of experiments[14] showed that the maximum in the curve k–M was shifted to higher M values if the column set contained gels with larger pores. Similarly, the curve k–M can be extended to lower molecular masses when using columns separating efficiently in the low M range. On the other hand, very low k values were observed[15] for M higher than the upper separation limit of the given g.p.c. system.

The above results show that the concentration effects in g.p.c. with rigid gels are connected mainly with the permeation of gel pores by solute molecules. However, if only mutual interactions of solute molecules and their shrinkage with the increasing C_i were responsible for con-

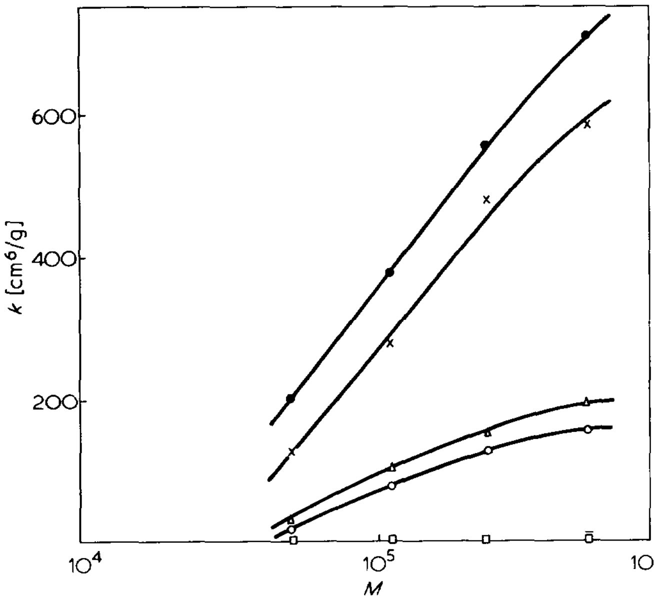

Figure 4 Plots of slopes k of V_e–C_i dependences from *Figure 3* vs. PS mol masses M in THF (●) and in binary eluents THF–water containing 4.5 (X); 7.7 (△); 8.2 (O); and 8.9 (□) vol % of water

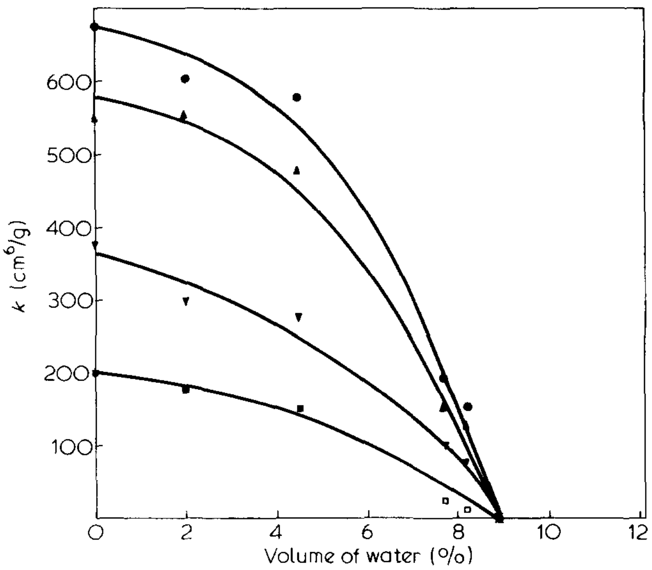

Figure 5 Plots of slopes k of V_e–C_i dependences from *Figure 3* vs. eluent composition for PS molecular masses M = 4.98 x 10^5 (●); 2.33 x 10^5 (▲); 1.1 x 10^5 (▼); and 5.1 x 10^4 (■)

centration effects, k would be zero in theta systems where macromolecules have their smallest dimensions.

The non-zero values of k in theta mixtures as well as for M larger than the excluded molecular mass indicate that at least one additional mechanism is also involved in the g.p.c. concentration effects with rigid gels. It may be, for example, the 'osmotic balancing of concentrations' proposed by Schweiger and Langhammer[16] or viscosity effects treated in detail by Janča and Pokorný[17]. However, the observation that the k values tend to reach zero in eluents poorer than theta mixtures indicates that the viscosity effects are not very important over the studied concentration (and viscosity) range.

Figure 5 shows the dependence of k on the eluent composition. The shape of the curves resembles changes of the limiting viscosity numbers and the polymer–solvent interaction parameters B with thermodynamic quality of the mixtures of THF and water (see *Figures 2* and *4* in ref 1). The variations of this type give a rough estimate of theta composition from g.p.c. measurements.

Previously[11] the common dependence of A_2M on k was found for various PS–eluent systems (A_2 is the second virial coefficient). This variation allows the estimation of A_2 values from g.p.c. data. The A_2 values for the systems PS–THF–water were not known; we calculated them by interpolation of Figure VIII–17 in ref 18 from limiting viscosity numbers of PS in THF–water mixtures. In addition, we used the viscosity interaction parameter B obtained[1] from Stockmayer–Fixman plots[18] for the correlation of the above type. The plots of A_2M and BM *versus* k are shown in *Figure 6*. Their courses are similar and curves do not pass through the origin. Evidently, the values of the A_2 coefficient (B parameter) and its change with water content in THF depend on the nature of polymer. Thus k will also change with water content in a different way for each polymer.

The situation with organic gels is expected to be similar. In the case of rigid gels V_e–C_i dependences and their changes with the water content on the one hand and with the nature and molecular mass of the polymers studied on the other hand will be comparable with those observed with inorganic gels. In the case of soft organic gels the situation will be further complicated by the effects of osmotic deswelling of the gel[19].

Calibration curves

A simple inspection of the concentration dependences in *Figure 3* shows that the course of calibration curves will depend on the injected polymer concentration. Since the extent of the concentration effects is generally a function of solvent 'goodness'[11], it is necessary to compare calibration curves at V_{e_0}, i.e. at V_e corresponding to zero polymer concentration.

Figure 7 represents the calibration curves $\log M = f(V_{e_0})$ for the systems THF–water. Curves A and B in *Figure 7* were obtained with the column system I; the V_{e_0} values in the linear region of the calibration curve were obtained by extrapolating V_e–C_i dependences to $C_i = 0$. $V_{e_0} = V_e$ at C_i = 1 mg/cm^3 were used for PS standards with $M >$ 6.7 x 10^5 and $M <$ 5.1 x 10^4 since the values for k in these cases are low as mentioned above. For the sake of clarity *Figure 7* contains only two curves (A and B) for the g.p.c. system I–namely those for dry THF and for a mixture containing 8.9% of water. Experimental points for other

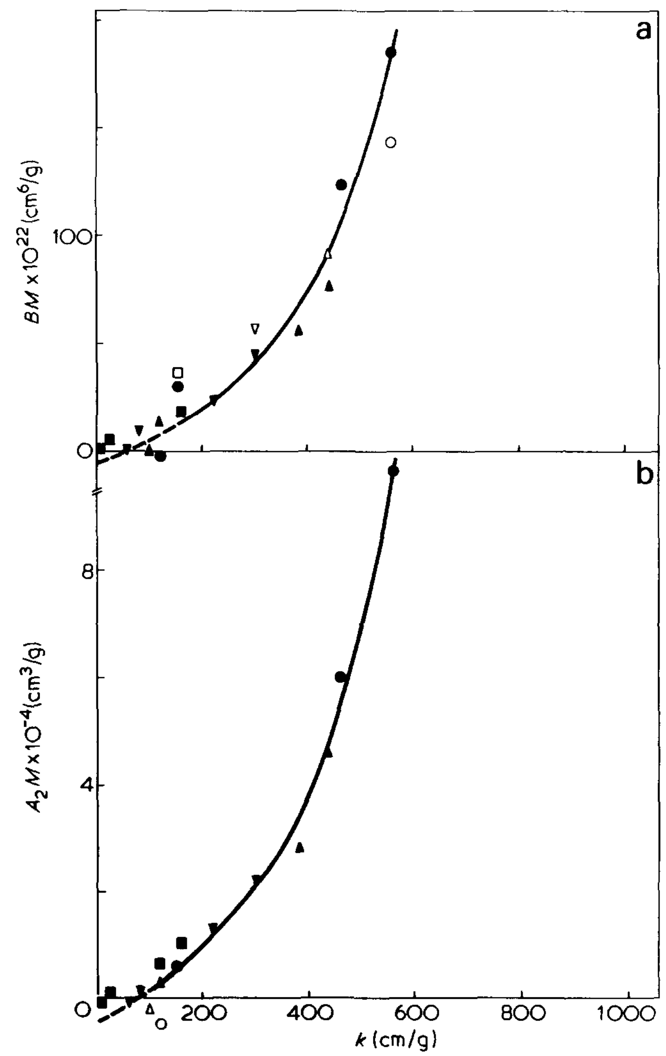

Figure 6 Correlation of slopes k of V_e–C_i dependences with the product of the second virial coefficients and molecular masses A_2M (a) and with the product of parameter B from Stockmayer–Fixman plot and mol. masses BM (b). The points are denoted as in *Figure 5*. A_2 values were either calculated according to ref 18 (full points) or taken from ref. 30 (empty points)

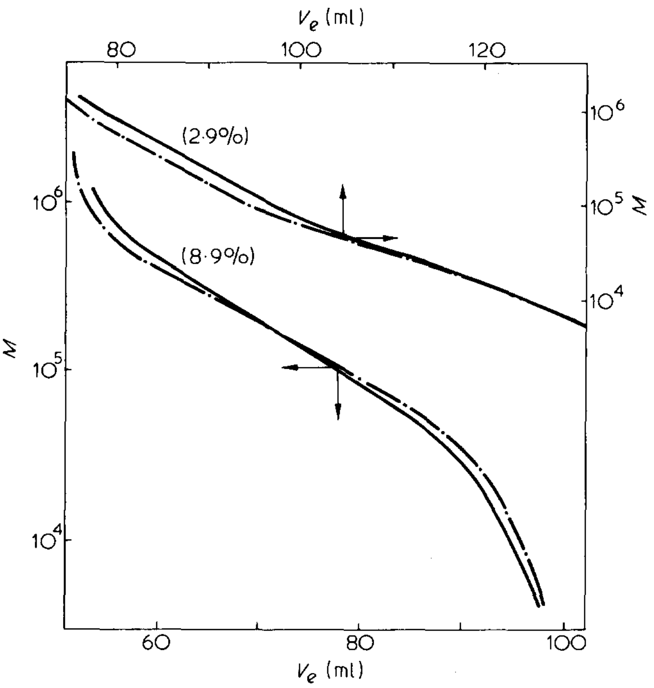

Figure 7 Calibration dependences log M–V_e for PS in THF (–·–·) and in mixtures (——). The curves (a) were obtained with the column system I, curves (b) with the system II. Numbers in brackets indicate % of water in binary eluent. –·–·–, THF; ——, THF–water

mixed eluents lay between curves A and B and were omitted. The courses of the calibration curves log M– V_e depend on the amount of water present in tetrahydrofuran and one can expect substantial errors in analytical g.p.c. data if there is a difference between the composition of eluent used for calibration and that employed for actual g.p.c. characterization of a polymer.

The extent of these errors increases with increasing polymer concentration, C_i, and the shift of V_e to higher values is more pronounced in pure THF than in mixed eluents THF–water (see *Figure 3*). This is evident from *Figure 7* curves C and D where the calibration curves are shown for dry THF and THF containing 2.9 vol % of water in System II at C_i = 2.0 mg/cm^3. The effects mentioned will further increase with the increasing chromatographic selectivity of the g.p.c. system used.

In order to account for the changes in coil dimensions when the eluent contains an increasing amount of water, hydrodynamic volumes of coils governing the actual size exclusion of solutes[20] should be compared rather than their molecular masses. The plots of log ([η]M) against V_e for the systems containing 0, 4.5, 7.7, 8.2 and 8.9% of water are given in *Figure 8*. It should be noticed that the limiting viscosity numbers for constructing the universal calibration curve (UCC) for solvent containing 8.9% of water were obtained by extrapolation of *Figure 2* in ref 1. The curves are shifted to lower V_e as the water content increases. In our opinion, adsorption and partition effects are responsible for these shifts, similarly as in the case of some other mixed eluents[10,21,22].

The role of solvent in g.p.c. adsorption effects can be better understood by comparison with liquid–solid chromatography (l.s.c.). The eluting power of an eluent in l.s.c. can be expressed by the so-called 'solvent strength parameter'[2]. THF belongs to solvents with the medium strength in this classification. On the other hand, water is one of the strongest solvents and is often used as a moderator in l.s.c. with polar sorbents and non-polar eluents. The role of the moderator is to block active sites of sorbent and to decrease the corresponding V_e. Generally, the more moderator the l.s.c. eluent contains the lower are the elution volumes of solutes under otherwise identical conditions.

The extent of polymer adsorption is further influenced by changing thermodynamic interactions between polymer and eluent[23] and, finally, the molecules of water adsorbed on the surface of the gel decrease the effective dimensions of the pores accessible to PS molecules and decrease the corresponding elution volumes.

The role of partition can now be considered. The concentration of water may increase in the whole pore volume. Consequently, the composition of the quasi-stationary phase (i.e. eluent in pores of the gel) differs from the eluent composition. This may cause the thermodynamic partition of polystyrene between both phases and a further change in V_e. Generally, the elution volumes should increase if the quasi-stationary phase is a thermodynamically better solvent than the mobile phase for solute and *vice versa*.

The quantitative and *a priori* description of the adsorption and partition effects would substantially improve the precision of g.p.c. measurements. However, this is rather difficult because we are not able to separate particular contributions. Supposing that Benoit's concept of universal calibration holds for the pure steric exclusion mechanism of separation and that limiting viscosity numbers are precise enough to describe the hydrodynamic volume of polymer coils. The summary g.p.c. secondary effects can be expressed in terms of V_e shifts under these conditions.

Dawkins and Hemming introduced[24] a distribution coefficient K_P involving all kinds of secondary mechanisms caused by interactions of solute–gel–eluent in the g.p.c. column. Then we have for V_e:

$$V_e = V_0 + K_D K_P V_i \qquad (2)$$

where V_0 is the interparticular (dead) volume and V_i the pore volume of the column; K_D stands for the distribution coefficient of the solute between the quasi-stationary and

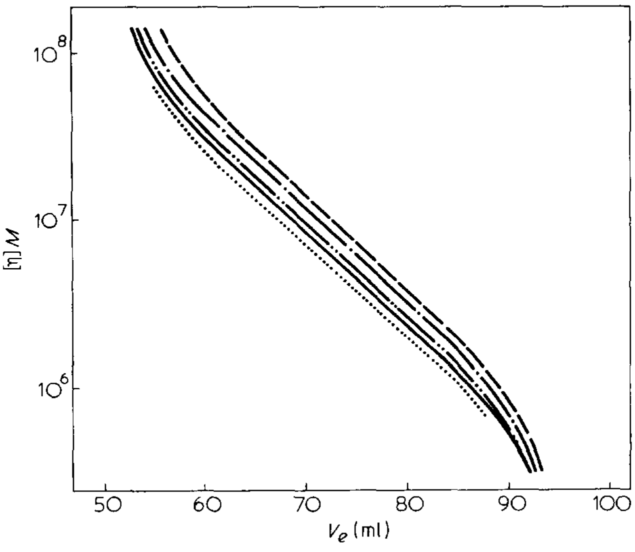

Figure 8 Universal calibration curves log[η] M–V_e obtained in the column system I in THF (–––) and in mixed eluents THF–water containing 4.5 (–·–·); 7.7 (–··–); 8.2 (——); and 8.9 (······) vol % of water

Table 1 K_P values from *Figure 8**

$[\eta] M \times 10^{-6}$	THF	THF–water 4.5%	THF–water 7.7%	THF–water 8.2%
30	1.72	1.54	1.28	1.15
11	1.34	1.25	1.13	1.07
5	1.24	1.18	1.09	1.06
2	1.18	1.13	1.07	1.04

*Mixture containing 8.9% of water was considered as the base for calculating K_P values

Table 2 Shifts of universal calibration curves from *Figure 8* and total solubility parameters for mixed solvents THF–water

Solvent	ΔV_{euc} (ml) for $[\eta] M = 1.1 \times 10^7$	δ_T (MPa$^{1/2}$)
THF	5.1	20.2
THF–water 4.5%	3.8	21.1
THF–water 7.7%	2.0	21.9
THF–water 8.2%	1.1	22.1
THF–water 8.9%	0	22.3
Water	–	52.2

mobile phases for the pure steric exclusion g.p.c. mechanism ($0 \leqslant K_D \leqslant 1$).

In the linear part of the calibration dependence:

$$K_D = -A \log[\eta] M + B \tag{3}$$

where A and B are constants. Combination of equations (2) and (3) gives:

$$\frac{V_e - V_0}{K_P} = V_i(-A \log[\eta] M + B) \tag{4}$$

By means of equation (4), Dawkins and Hemming[24] succeeded in correcting their g.p.c. data for secondary effects in the systems polystyrene gel–poor solvents–PS solute. Recently Dawkins claimed[25] that equation (4) accounts for solute–gel interactions also in rigid inorganic packings. We tried to apply equation (4) to our data but we found the pronounced dependence of K_P on polymer molecular mass (see *Table 1*), which complicates practical use of Dawkins and Hemming original procedure[24] or its modifications[26].

The attempt was made[21] to describe the adsorption and partition effect *a priori*. We can write:

$$V_e = V_0 + \Delta V^E{}_e + \Delta V^S{}_e + \Delta V^P{}_e + \cdots \tag{5}$$

where $\Delta V^E{}_e$ is the exclusion contribution and similarly $\Delta V^S{}_e$ is the adsorption and $\Delta V^P{}_e$ the partition contribution to the elution volume. It was shown[21] that $\Delta V^S{}_e$ and $\Delta V^P{}_e$ can approximately be expressed in terms of the solubility parameters of eluent components and polymer.

The values of $\Delta V_{euc} = \Delta V^S{}_e + \Delta V^P{}_e$ at $[\eta] M = 1.1 \times 10^7$ (for $[\eta]$ in cm^3/g) together with the solubility parameters of mixed eluents are given in *Table 2*. The values for V_e in THF containing 8.9% of water were chosen as a base for calculating ΔV_{euc}. The total solubility parameters δ_T of mixed solvents were calculated according to equation (16) and data from *Table 1* in ref 27 using linear interpolation of component solubility parameters of THF and water.

The data in *Table 2* show that the shifts in universal calibration curves ΔV_{euc} can be correlated with solubility parameters of mixed eluents THF–water. The dependence of $\Delta V_e^{S,P}$ on the solubility parameter of mixed eluent THF–water is similar to that found for other mixed eluents[21] and correlations of this type can be used for estimating the shifts of calibration curves with the water content of the eluent.

Both adsorption and partition are quite general secondary mechanisms in gel chromatography with mixed eluents. Effects of adsorption are more important with polar column filling materials like porous glass, silica gel, poly(vinyl acetate) or dextran. Partition may play quite an important part with gels having less smooth and defined pore walls, i.e. with organic gels regardless of their polarity. Evidently, the extent of adsorption and partition also depends on solute type and concentration of water in the eluent. The changes in elution volumes resulting from adsorption and partition effects cannot be eliminated using universal calibration plots or by extrapolating V_e to zero injected polymer concentration. Erroneous g.p.c.-based data of $\bar{M}_w$ and $\bar{M}_n$ are thus obtained not only when the amount of water in the eluent changes but also when g.p.c. chromatograms of polar polymers obtained with humid eluent are evaluated using UCC measured with narrow molecular weight distribution polystyrenes.

The above observations can be generalized further. Instead of water, other admixtures can be present in the eluent. Generally, the shifts of UCC will increase with increasing differences between the polarity of admixture on the one hand and the polarities of eluent, separated polymer and gel on the other hand.

CONCLUSIONS

It has been shown that the presence of water in the g.p.c. eluent–tetrahydrofuran–may influence:

(1) the shape of the chromatograms. New peaks are produced at high elution volumes and the shape of solute peaks is also changed;

(2) the course of the dependence of elution volume on concentration;

(3) the extent of the secondary separation mechanisms such as adsorption and partition.

The above phenomena together with changes of hydrodynamic volume of the macromolecules due to the presence of water lead to shifts in g.p.c. elution volumes and cause substantial errors in gel chromatographic results obtained with humid THF, especially if the water content changes. These effects were demonstrated with the SiO_2 column packing materials and narrow polystyrenes; however, the results can be generalized to other gels and separated polymers and even to other admixtures in the eluent. The application of low molecular internal standards as proposed by Patel[28] cannot help to solve the above problems because several effects described in this paper are effective for macromolecular substances only and their extent depends on both the molecular mass and the chemical composition of the solute.

Careful and standard purification of THF together with its analytical control is experimentally rather difficult. The use of THF containing a constant amount of water (e.g. azeotropic mixture) would need corresponding corrections ($[\eta]$ values, V_e shifts for different polymers, etc.). Both procedures suffer from problems connected with the protection of the eluent from moisture and prevention from its composition changes during application.

The requirement of the extremely dry eluent may be fulfilled by using guard columns put in g.p.c. systems just before or behind the pumping system. A guard column of appropriate size can be filled with activated silicagel or with a molecular sieve. Its use would be justified especially in high performance g.p.c. where high precision is needed and elution volumes are small. Our preliminary experiments[29] showed that the presence of water in the injected polymer solution influenced the observed V_e much less than water present in the eluent. This means that the solutions of the polymers analysed could be prepared without severe protection against humidity.

REFERENCES

1 Spychaj, T., Lath, D. and Berek, D. *Polymer* 1979, **20**, 437
2 Snyder, L. R. 'Principles of Adsorption Chromatography', Marcel Dekker, New York, 1968
3 Thomas, J. P., Brun, A. and Bounine, J. P. *J. Chromatogr.* 1977, **139**, 21
4 Šlais, K. and Krejčí, M. *J. Chromatogr.* 1974, **91**, 161
5 Berek, D., Bleha, T. and Pevná, Z. *J. Chromatogr. Sci.* 1976, **14**, 560
6 Solms, D. J., Smuts, T. W. and Pretorius, V. *J. Chromatogr. Sci.* 1971, **9**, 600
7 Hert, M., Strazielle, C. and Benoit, H. *Makromol. Chem.* 1973, **172**, 169
8 Hert, M. and Strazielle, C. *Makromol. Chem.* 1974, **175**, 2149
9 Ely, D. D. *J. Polym. Sci. (C)* 1968, **21**, 13
10 Berek, D., Bakoš, D., Bleha, T. and Šoltés, L. *Makromol. Chem.* 1975, **176**, 391
11 Bleha, T., Bakoš, D. and Berek, D. *Polymer* 1977, **18**, 897
12 Dondos, A. and Benoit, H. *J. Polym. Sci. (B)* 1969, **7**, 335
13 Bakoš, D., Berek, D. and Bleha, T. *Eur. Polym. J.* 1976, **12**, 801
14 Spychaj, T. *PhD Thesis* Polymer Institute of the Slovak Academy of Sciences, Bratislava, 1978
15 Vondra, R. *Thesis* Polymer Institute of the Slovak Academy of Sciences, Bratislava, 1975
16 Schweiger, M. and Langhammer, G. *Plast. Kautsch.* 1977, **24**, 101
17 Janča, J. *J. Chromatogr.* 1977, **134**, 263; Janča, J. and Pokorný, S. *J. Chromatogr.* 1978, **148**, 31
18 Yamakawa, H. 'Modern Theory of Polymer Solutions', Harper and Row, New York, 1971, Ch. 7, p. 384
19 Baghurst, P. A., Nichol, L. W., Ogston, A. G. and Winzor, D. J. *Biochem. J.* 1975, **147**, 575
20 Grubisic, Z., Rempp, P. and Benoit, H. *J. Polym. Sci. (B)* 1967, **5**, 753
21 Bakoš, D., Bleha, T., Ozimá, A. and Berek, D. *J. Appl. Polym. Sci.* in press
22 Campos, A., Borque, L. and Figueruelo, J. E. *J. Chromatogr.* 1977, **140**, 219
23 Spychaj, T., Berek, D., Bleha, T. and Vondra, R. presented at 'Chromatography of Polymers and Polymers in Chromatography', Prague, 1978
24 Dawkins, J. V. and Hemming, M. *Makromol. Chem.* 1975, **176**, 1796
25 Dawkins, J. V. paper presented 'Advances in the Chromatographic Fractionation of Macromolecules', University of Birmingham, 1976
26 Campos, A. and Figueruelo, J. E. *Makromol. Chem.* 1977, **178**, 3249
27 Tijssen, R., Billiet, H. A. H. and Schoenmakers, P. J. *J. Chromatogr.* 1976, **122**, 185
28 Patel, G. N. and Stejny, J. *J. Appl. Polym. Sci.* 1974, **18**, 2069
29 Berek, D. and Figueruelo, J. E. paper presented at 'Chromatography of Polymers and Polymers in Chromatography', Prague, 1978
30 Quano, A. C. *J. Chromatogr.* 1976, **118**, 303