Non-exclusion phenomena in size exclusion chromatography with inorganic porous packing: a thermodynamic treatment

Rosa García, Bernardo Celda, Vicente Soria, Roberto Tejero and Agustín Campos*

Departamento de Química Física, Facultad de Químicas, Universitat de Valencia, Burjassot, Valencia, Spain

(Received 20 April 1989; revised 4 December 1989; accepted 11 December 1989)

Diverse theoretical expressions have been used to describe the deviations in solute retention in size exclusion chromatography (s.e.c.) with mixed eluents. Most of them are based on the Flory–Huggins (FH) thermodynamic model of polymer solutions. Recently, and using a modified FH theory, we have calculated distribution coefficients K_p, in s.e.c. with organic packings. A thermodynamic extension of FH formalism to a rigid gel (i.e. silica-based) in a binary solvent has allowed us to describe the elution mechanisms of a polymer molecule in these systems. The approach used is based on the determination of the polymer concentration ratio between the mobile and quasi-stationary phases, taking into account exclusively FH binary interaction parameters between polymer solute and two components of the eluent. The contribution of preferential solvation of the polymer at a given solvent (eluent) composition has also been analysed. This assumption has been verified using chromatographic data of different polystyrene standards in benzene–methanol, butanone–heptane and carbon tetrachloride–methanol on Spherosil as column packing. Assuming a cylindrical profile for the pores and considering the influence of the preferential solvation of the polymer in each phase, a good correlation between experimental and predicted values of K_p has been obtained.

(Keywords: distribution coefficient; Spherosil; polystyrene; h.p.l.c.–s.e.c.; Flory–Huggins theory; interaction parameters; ternary polymeric systems)

INTRODUCTION

Hydrodynamic volume $[\eta]M$, where $[\eta]$ and M are the intrinsic viscosity and molecular weight of a polymeric sample, respectively, or a size parameter related to this volume such as the radius or diameter of the hydrodynamic sphere, is a universal calibration parameter in size exclusion chromatography (s.e.c.) for synthetic homopolymers and copolymers having linear and branched structures[1–3]. When the organic packing material is cross-linked polystyrene gel and the mobile phase is compatible with the polystyrene gel, and with the polymer solute, the first condition being more important, solutes do not display preferential affinity or preferential adsorption for the mobile or stationary phase. Hence, partition and adsorption mechanisms do not influence the solute size separation[4]. However, when the solute polymers are eluted in poor or theta solvents for solute and gel (i.e. cyclohexane at 35°C with polystyrene solute and polystyrene gel) solute–gel interactions become important and secondary mechanisms appear (adsorption and partition[5–7]) with the elution volume, V_R, being displaced to high V_R values with respect to universal calibration. When there is incompatibility between solute polymer and cross-linked polystyrene gel, V_R values are shifted toward smaller retention volumes[8]. For these systems the values of the partition coefficient, K_p, have been theoretically obtained as a function of Flory–Huggins' interaction parameters[8,9]. Likewise, from a theoretical point of view, K_p has been related with the parameter of preferential sorption, λ, of the gel in solvent–eluent/polymer–solute/cross-linked-polymeric gel ternary systems, with the evaluation of its volume fraction solving an important difficulty for K_p prediction[8,10].

The s.e.c. behaviour of synthetic polymers in good and theta solvents has also been compared using polar inorganic packings (porous silica)[11,12]. For these systems observed deviations with respect to the universal calibration, UC (UC is usually obtained using narrow polymer samples with different chemical composition in good solvents[13]) are practically independent of the thermodynamic quality or compatibility of the eluent for the polymeric solute or gel, respectively. The deviations appear when simultaneously the interactions between the solvent and gel and between solvent and polymer solute, are markedly modified by using a mixture of two solvents as eluent. Due to the preferential sorption (as a consequence of specific interactions) of the gel by one of the components of the mixed eluent, a difference in the thermodynamic quality of the mobile and quasi-stationary phases appears and consequently, additional partition of the solute may occur[12]. In these cases, and in the case of organic gels like cross-linked polystyrene, K_p can be theoretically defined as the ratio between volume fraction of polymer solute in the two phases, ($K_p = \phi_3/\phi'_3$), mobile and quasi-stationary both with different mixture composition of the solvent–eluent, when the equilibrium is reached.

---

* To whom correspondence should be addressed

0032-3861/90/091694-09

The aim of this work is to demonstrate that the deviations of elution volumes attributed to K_p in s.e.c. behaviour using polar inorganic packing can be explained through thermodynamic equations for the distribution coefficient of polymer between the two ternary phases, (in which the interaction parameters of Flory–Huggins between the polymer solute and two components of the eluent are exclusively taken into account). Any direct magnitude referring to the gel is excluded from these equations. Nevertheless, the gel is indirectly considered, because the evaluation of the composition of the binary solvent in the quasi-stationary phase is obtained from adsorption isotherms in which the adsorbate is the polar component of the solvent mixture and the adsorbent is the gel[14]. The expressions obtained have been verified in the ternary polymer systems (TPS) benzene(Bz)/methanol(M)/polystyrene(PS), butanone(MEK)/heptane (Hep)/PS, and carbon tetrachloride(CTC)/M/PS using Spherosil as column packing. The influence of preferential solvation of the polymer by one of the components of the binary solvent mixture, λ, at a given eluent (solvent) composition has been also analysed. The best fit between experimental and predicted values of K_p was obtained when a cylindrical profile for the pores was assumed and λ influence was considered.

THERMODYNAMIC CALCULATIONS

A s.e.c. polymer fractionation with an inorganic packing when the eluent is a binary solvent mixture can be regarded as a TPS constituted by solvent(1)/solvent(2)/polymer solute(3). Under the usual chromatographic conditions and from a thermodynamic point of view this system can be considered as being formed by two phases coexisting in equilibrium. The first is a 'mobile phase' constituted by the polymer solution outside the pore or inside the pore but away from the pore walls. The second is a 'quasi-stationary phase' constituted by the mixture of polymer and solvents neighbouring pore walls.

Owing to the specific interactions between the gel by one of the components of the mixed eluent, a difference in the composition of the mobile phase and quasi-stationary phase is carried out, for instance, when methanol, in a benzene–methanol mixture, is bonding via hydrogen bonding with silanol groups present at the surface of the gel.

Throughout this paper, the following nomenclature will be used to define the composition in both phases:

ϕ_i ($i=1,2,3$) is the volume fraction of the component i in the quasi-stationary phase ($\sum \phi_i = 1$).
ϕ'_i ($i=1,2,3$) is the volume fraction of the component i in the mobile phase ($\sum \phi'_i = 1$). u_i and u'_i ($i=1,2$) are the volume fractions of the solvent mixture in both the quasi-stationary and mobile ternary phases, respectively; $u_i = \phi_i/(1-\phi_3)$ and $\sum u_i = 1$.

The Flory–Huggins equation as modified by the Pouchly expression for the Gibbs free energy of mixing, ΔG_M, for a ternary system formed by two solvents and one polymer is[15,16]

$$\frac{\Delta G_M}{RT} = \sum_{i=1}^{3} n_i \ln \phi_i + n_1\phi_2 g_{12}(u_1) + n_1\phi_3 g_{13}(\phi_3) + n_2\phi_3 g_{23}(\phi_3) + n_1\phi_2\phi_3 g_T(u_1, \phi_3) \tag{1}$$

where n_i is the number of moles of component i in the mixture, g_{ij} and g_T the Flory–Hugginss' binary and ternary interaction parameters concentration dependences, which are related to the classical parameters, χ_{ij} and χ_T by[17,18]

$$\chi_{ij}(\phi_3) = g_{ij}(\phi_3) - (1-\phi_3)(\mathrm{d}g_{ij}/\mathrm{d}\phi_3) \tag{2}$$

$$\chi_T(u_1, \phi_3) = g_T(u_1, \phi_3) - (1-\phi_3)(\partial g_T/\partial\phi_3)_{u_1} \tag{3}$$

Following the nomenclature of Pouchly *et al.*[19,20]

$$G_u = \frac{RT}{V_1}\left[u_1 \ln\frac{u_1}{1+u_3} + su_2 \ln\frac{u_2}{1+u_3} + \frac{V_1}{V_3} u_3 \ln\frac{u_3}{1+u_3} + \frac{u_1u_2}{1+u_3} g_{12} + \frac{u_1u_3}{1+u_3} g_{13} + \frac{su_2u_3}{1+u_3} g_{23} + \frac{u_1u_2u_3}{(1+u_3)^2} g_T\right] \tag{4}$$

where $G_u = \Delta G_M/V(1-\phi_3)$, $u_i = \phi_i(1+u_3)$, V_i is the molar volume of component i, V is volume total of the mixture and $s = V_1/V_2$.

The chemical potential of the polymer in the ternary mixture may be defined as:

$$\left(\frac{\partial G_u}{\partial u_3}\right)_{u_1} = \frac{RT}{V_1}\left[-\frac{u_1}{1+u_3} - \frac{su_2}{1+u_3} + \frac{V_1}{V_3}\left(\ln\frac{u_3}{1+u_3} + \frac{1}{1+u_3}\right) - \frac{u_1u_2}{(1+u_3)^2} g_{12} + \frac{u_1}{(1+u_3)^2} g_{13} + \frac{u_1u_3}{1+u_3}\left(\frac{\mathrm{d}g_{13}}{\mathrm{d}u_3}\right) + \frac{su_2}{(1+u_3)^2} g_{23} + \frac{su_2u_3}{1+u_3}\left(\frac{\mathrm{d}g_{23}}{\mathrm{d}u_3}\right) + \frac{u_1u_2(1-u_3)}{(1+u_3)^3} g_T + \frac{u_1u_2u_3}{(1+u_3)^2}\left(\frac{\mathrm{d}g_T}{\mathrm{d}u_3}\right)\right] \tag{5}$$

As is generally assumed in liquid chromatography the chemical potential of the polymer is the same in the mobile and quasi-stationary phases when the equilibrium between both ternary phases is attained, then:

$$\begin{aligned} &-\phi_1 - s\phi_2 + \frac{V_1}{V_3}[\ln\phi_3 + 1 - \phi_3] - \phi_1\phi_2 g_{12} + \phi_1(1-\phi_3)g_{13} + \frac{\phi_1\phi_3}{1-\phi_3}\left(\frac{\mathrm{d}g_{13}}{\mathrm{d}\phi_3}\right) + s\phi_2(1-\phi_3)g_{23} \\ &+ \frac{s\phi_2\phi_3}{1-\phi_3}\left(\frac{\mathrm{d}g_{23}}{\mathrm{d}\phi_3}\right) + \phi_1\phi_2(1-2\phi_3)g_T + \frac{\phi_1\phi_2\phi_3}{1-\phi_3}\left(\frac{\partial g_T}{\partial\phi_3}\right)_{u_1} \\ &= -\phi'_1 - s\phi'_2 + \frac{V_1}{V_3}[\ln\phi'_3 + 1 - \phi'_3] - \phi'_1\phi'_2 g_{12} + \phi'_1(1-\phi'_3)g_{13} + \frac{\phi'_1\phi'_3}{1-\phi'_3}\left(\frac{\mathrm{d}g_{13}}{\mathrm{d}\phi_3}\right) + s\phi'_2(1-\phi'_3)g_{23} \\ &+ \frac{s\phi'_2\phi'_3}{1-\phi'_3}\left(\frac{\mathrm{d}g_{23}}{\mathrm{d}\phi_3}\right) + \phi'_1\phi'_2(1-2\phi'_3)g_T + \frac{\phi'_1\phi'_2\phi'_3}{1-\phi'_3}\left(\frac{\partial g_T}{\partial\phi_3}\right)_{u_1} \end{aligned} \tag{6}$$

From a theoretical point of view, the expression (6) is completely valid for K_p prediction[8,10], both for systems with inorganic and with organic packings. However, in the last system the gel fraction solvated by the polymeric solution is not accessible from experiment, hence the exact evaluation of K_p is not possible.

Alternatively, it follows that the retention volume V_R of a macromolecule substance can be written as

$$V_R = V_0 + K_{\text{s.e.c.}} V_p \qquad (7)$$

where $K_{\text{s.e.c.}}$ is formally analogous to the distribution coefficient[21]. V_0 is the interstitial or void volume and V_p is the total volume of solvent within the gel. On the other hand, Dawkins[5,7] defined $K_{\text{s.e.c.}}$ as:

$$K_{\text{s.e.c.}} = K_D K_p \qquad (8)$$

where K_D is the distribution coefficient for pure size exclusion and K_p is the additional distribution coefficient for solute–gel (cross-linked polystyrene porous packing) interaction effects[22], or additional partition distribution coefficient for selective dissolution of solute in mobile and stationary phases (inorganic packing) caused by selective sorption of one component of mixed solvent on porous silica[12]. The term K_D in equation (8) may be expressed as a function of hydrodynamic volume:

$$K_D = -A \log [\eta] M + B \qquad (9)$$

where A and B are constants[4,22]. Substitution of equations (8) and (9) into equation (7) and rearrangement gives:

$$\log [\eta] M = \frac{B}{A} - \frac{1}{A V_p} \frac{V_R - V_0}{K_p} \qquad (10)$$

A plot of $\log [\eta] M$ vs. $(V_R - V_0)/K_p$ will give a universal calibration when solutes are exclusively separated by size exclusion ($K_p = 1$), but also when solutes are separated by size exclusion and other secondary mechanisms (with mixed solvent and porous silica, partition by different solubility: $K_P \neq 1$).

RESULTS AND DISCUSSION

From an experimental point of view, the distribution coefficients K_p have relative characters and their empirical evaluation requires a reference system in which $K_p = 1$ (pure size exclusion). For organic gels the selection of an experimental reference system presents some difficulties, mainly due to not having a precise definition of their properties, i.e. the mobile phase must be very compatible with the gel and with solute polymers[4], the first condition being more important. In addition, for these systems the choice of a theoretical reference system shows problems as well. $K_{\text{s.e.c.}}$ may be defined as the product of two exponential terms, enthalpic and entropic, the former usually being considered like a distribution coefficient[22,23]. Therefore, as the reference system implies that size exclusion is the only type of mechanism governing the solute separation, the exponential enthalpic term must be unity, and hence V_R must be invariant with the temperature[22,23]. There are other ways to find a theoretical reference system, $K_p = 1$, but in all of them it would be necessary to know g_{ij} parameters and the solvated gel fraction by the polymeric solution during the process of separation, which are not easily accessible by experiment[8,10].

However, for silica-based inorganic gels the selection of a reference system does not cause any special difficulty, if the secondary mechanism of partition (by different solubility of polymer solute between mobile and quasi-stationary phases) is taken into account by discarding the adsorption mechanism. Therefore, the mobile phase for the reference systems in inorganic gels will be formed by one pure solvent, compatible with the solute and with the gel. Thus the partition effect is avoided and the polymer solute is only separated by size exclusion[13]. Likewise, from a theoretical point of view these systems can be considered as reference systems, because the calibration curves $\log [\eta] M$ vs. V_R in eluents formed by a pure solvent must be independent of the temperature, which has been experimentally verified[24], then $K_p = 1$ (ref. 22).

Two facts make inorganic gel systems ideal for the comparative study of K_p theoretical values obtained from equation (8) and experimental values. On the one hand, the selection of the reference system does not present any special problem[13], but on the other hand, in certain conditions exclusively partition mechanisms appear and adsorption secondary mechanisms do not. This comparative analysis can be carried out by using the theoretical K_p values in the plot $\log [\eta] M$ vs. $(V_R - V_0)/K_p$; hence the secondary mechanisms are avoided when $K_p \neq 1$, and a UC may be obtained.

The prediction of K_p through equation (6) requires extensive and homogeneous experimental information, and we made use of the results from our laboratory[13,14,25]. The systems studied were: Bz(1)/M(2)/PS(3) in Bz–M mixtures: $\phi_{10} = 0.75$, 0.84, 0.90 and 0.99; CTC(1)/M(2)/PS(3) in CTC–M mixtures: $\phi_{10} = 0.85$, and MEK(1)/Hep(2)/PS(3) in mixtures MEK–Hep: $\phi_{10} = 0.75$, 0.60, 0.50 and 0.40. Where ϕ_{i0} is the volume fraction of component i in the original binary mixture, ϕ_{i0} is usually known as 'bulk solvent' composition, and in this case stands for the composition of the eluent.

In order to simplify the discussion of results, let us describe the procedure of calculation of the different magnitudes in equation (6). This description has been divided following the structure of that equation. Hence the magnitudes corresponding to the mobile phase or second member of equation (6) will be discussed first. Next the magnitudes corresponding to the polymeric fraction in the quasi-stationary phase, or first term of that expression, will be discussed.

Magnitudes corresponding to the mobile phase

ϕ'_3. The value of ϕ'_3 depends on the concentration, c_3, and density of the polymer. For molecular weight less than 49 000, $c_3 = 3\,\text{mg ml}^{-1}$ (ref. 13) and $\phi'_3 = 2.77 \times 10^{-3}$. In the case of molecular weight higher than 49 000, $c_3 = 0$ (ref. 13) or experimental values of c_3 extrapolated to infinite dilution of the injected polymer concentration. In the first case it is assumed that during the chromatographic process there is no dilution of the injected solution[26]. In accordance with the experimental results, for polymer molecular weights higher than 49 000 the value of ϕ'_3 used was 10^{-10}–10^{-15}.

ϕ'_1 *and* ϕ'_2. In accordance to the nomenclature used $\phi'_i = (1 - \phi'_3) u'_i$, $(i = 1, 2)$ and $\sum \phi'_i = 1$. Two possible cases can be distinguished. First, in the absence of preferential solvation of the polymer for one of the components of the solvent mixture, $u'_i = \phi_{i0}$ $(i = 1, 2)$ (ref. 11) and

$$\phi'_i = (1 - \phi'_3) \phi_{i0} \qquad (i = 1, 2) \qquad (11)$$

Second, for the systems with preferential solvation the

Table 1 Values of the parameters needed for ϕ_3 evaluation, from equation (6)

System	g_{13}	g_{23}	χ_{13}	χ_{23}	a_g	a_χ	s	$\bar{V}_3$ (ml/g)
Bz/M/PS	0.455^a	0.973^a	0.455^a	0.723^b	0.97^b	$0.415^{a,b}$	2.195^a	0.923^a
CTC/M/PS	$0.45^{b,c}$	1.036^b	0.42^b	0.74^b	$0.89^{a,b}$	$0.47^{a,b}$	2.384^b	0.923^a
MEK/Hep/PS	0.65^f	1.22^h	1.22^f	1.12^h	1.114^c	0.911^d	0.609^e	0.923^a

For Bz/M/PS $g_{12}(\phi_{10})=2.5308+1.1068\phi_{10}-23.287\phi_{10}^2+210.05\phi_{10}^3-720.71\phi_{10}^4+1195.4\phi_{10}^5-956.13\phi_{10}^6+297.97\phi_{10}^7$ (see ref. 27)
For CTC/M/PS $g_{12}(\phi_{10})=2.632+0.03\phi_{10}+6.036\phi_{10}^2-9.274\phi_{10}^3+6.143\phi_{10}^4+3.98\phi_{10}^5$ (see ref. 28)
For MEK/Hep/PS $g_{12}(\phi_{10})=2.331-1427\phi_{10}-3.482\phi_{10}^2+9.568\phi_{10}^3-5.892\phi_{10}^4$ (see ref. 29)
[a] From ref. 15
[b] ref. 16
[c] ref. 17
[d] ref. 18
[e] ref. 29
[f] ref. 30
[h] ref 31
$(\mathrm{d}g_{i3}/\mathrm{d}\phi_3)=g_{i3}-\chi_{i3}$ $(i=1,2)$ and $(\mathrm{d}g_T/\mathrm{d}\phi_3)=2(a_g-a_\chi)g_{12}$

variation of volume fraction of component i in the ternary phase with respect to binary phase, may be defined by $\Delta\phi_{i0}=\phi_i-\phi_{i0}$. This value can be related to preferential solvation parameter, λ, through $\lambda=\Delta\phi_{i0}/c_3$. The gel permeation chromatography is a powerful technique for the experimental evaluation of λ (ref. 27), which has been used to determine it for some of the systems here discussed[25]. On the other hand, in these cases the composition of components 1 and 2 can be expressed by $u_1^\lambda=\phi_{10}+\Delta\phi_{10}$ and $u_2^\lambda=\phi_{20}-\Delta\phi_{10}$, respectively, if the polymeric solute is preferentially solvated by the component 1 of the solvent mixture. So, it can be written,

$$\phi_i'^\lambda=(1-\phi_3')u_i^\lambda=(1-\phi_3')(\phi_{i0}\pm\Delta\phi_{10})=\phi_i'\pm(1-\phi_3')\,\Delta\phi_{10} \qquad (i=1,2) \quad (12)$$

where $\Delta\phi_{10}=\lambda c_3$. The values of λ for each polymer molecular weight of PS and solvent mixture composition for the system Bz/M/PS and CTC/M/PS have been given elsewhere[25].

The values of binary and ternary Flory–Huggins' interaction parameters concentration dependences have been approximated at polymer infinite dilution as

$$g_{i3}(\phi_3)=g_{i3}^\circ+\phi_3\left(\frac{\mathrm{d}g_{i3}}{\mathrm{d}\phi_3}\right)_{\phi_3\to 0}\approx g_{i3}^\circ \qquad (i=1,2)$$

and

$$g_T(u_1,\phi_3)=g_T^\circ(u_1)+\phi_3\left(\frac{\partial g_T}{\partial\phi_3}\right)_{u_1,\phi_3\to 0}\approx g_T^\circ(u_1)$$

In *Table 1*, the values of these parameters, and the binary interaction potentials between both components of the solvent mixture, $g_{12}(\phi_{10})$, are gathered. The references, calculation procedure and approximations utilized are detailed in the footnote to the table.

Finally, the different magnitudes corresponding to the mobile phase or second term of equation (6) include the ratio between molar volumes of two solvents for each system, $s=V_1/V_2$, and the molar volume, $V_3=\bar{v}_3M_3$, where $\bar{v}_3$ is the polymer partial specific volume, $\bar{v}_3=0.923$ (ref. 3) for PS and M_3 is the molecular weight.

Magnitudes corresponding to the quasi-stationary phase or first term of equation (6)

ϕ_3. ϕ_3 is the polymer fraction in the ternary phase 'inside of the pore'. Its value is calculated using equation (6).

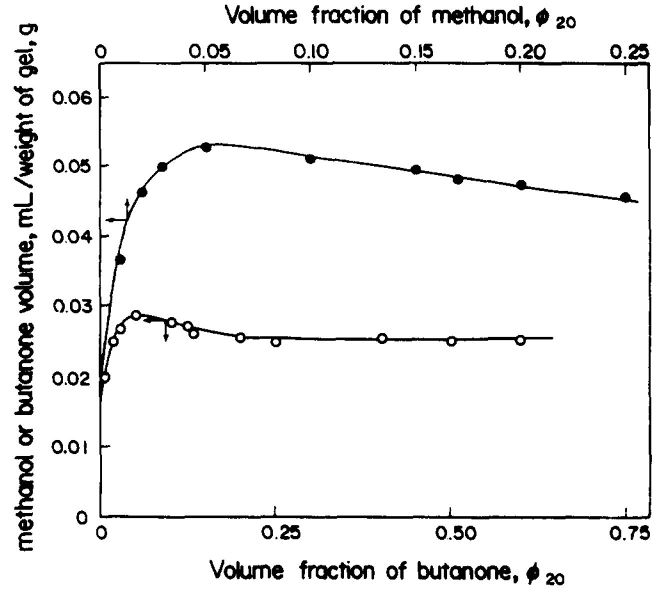

Figure 1 Relative methanol (●) or butanone (○) sorption on Spherosil XOA-200 of carbon tetrachloride–methanol and butanone–heptane mixtures

ϕ_i $(i=1,2)$. For the calculation of these values it is necessary to use isotherms of relative methanol sorption on Spherosil gel for Bz–M mixtures[14], and isotherms of relative butanone and methanol sorptions on Spherosil XOA-200 for MEK-Hep and CTC-M mixtures, respectively. These isotherms are shown in *Figure 1*. The physical meaning of those measurements was given elsewhere[14].

The composition of component 2 in the solvent mixture inside of the pores can be obtained through the values of volume fraction component 2 in the initial eluent, ϕ_{20}, and for the systems containing methanol with the corresponding isotherms. For a given value of ϕ_{20} the isotherm yields the volume in ml of component 2 adsorbed per g of gel. This value multiplied by 74 g (total amount of Spherosil XOA-200 gel used in all the experiments in this work[13] and divided by the volume of pores, V_p, gives the volume in ml of component 2 adsorbed per ml of pore volume. For the evaluation of V_p, or pore volume of gel free of any adsorbed substance, some assumption about the geometric shape of the cavity pore must be introduced. Thus, if it is assumed a spherical form,

$$V_p=V_p^{\mathrm{exp}}\left(\frac{\bar{r}}{r}\right)^3 \qquad (13)$$

and in the case of a cylindrical form,

$$V_{\mathrm{p}} = V_{\mathrm{p}}^{\mathrm{exp}}\left(\frac{\bar{r}}{r}\right)^2 \frac{H}{h} \tag{14}$$

where $V_{\mathrm{p}}^{\mathrm{exp}} = V_{\mathrm{m}} - V_0$, is the experimental pore volume for each eluent composition (*Table 1* in reference 13), $\bar{r}$ is the average pore radius given by the manufacturer ($\bar{r} = 75$ Å (ref. 13)) and r is the pore radius experimentally obtained. For the height of the clean pore cavity in the cylindrical shape it is assumed that $H = 2\bar{r}$. Whereas, the experimental value for this magnitude is supposed to be $h = \bar{r} + r$, which corresponds to the actual pore diameter accessible to the macromolecule under experimental conditions. On the other hand, it is interesting to point out that there are several values for r. So, for instance, in the case of maximum strength eluents as Bz–M (75:25), $\phi_{20} = 0.25$, or CTC–M (78:22), $\phi_{20} = 0.22$, $r = \bar{r}/3$. Obviously, the value of V_{p} calculated from equations (13) and (14) will depend on the different experimental values of r for each eluent mixture composition. In the footnotes of *Tables 2–6* the different values of $\bar{r}/r$ used in each case for each value of υ_2 (systems with methanol) and υ_1 (systems with butanone) as well as the references utilized are indicated.

The total volume, in ml, of component 2 per 1 ml of pores for a given eluent composition, ϕ_{20}, is defined by $[(1-\upsilon_2)\phi_{20} + \upsilon_2]$. In thermodynamic equilibrium conditions, this value can be considered equal to the composition of component 2 in the ternary solution passing through the inner part of the pore, then

$$\phi_2 = u_2(1-\phi_3) = [(1-\upsilon_2)\phi_{20} + \upsilon_2](1-\phi_3) \tag{15}$$

and

$$\phi_1 = [1 - (1-\upsilon_2)\phi_{20} - \upsilon_2](1-\phi_3) \tag{16}$$

or in a general case

$$\phi_i = [(1-\upsilon_i)\phi_{i0} + \upsilon_i](1-\phi_3) \tag{17}$$

if it is assumed that the component i is adsorbed on the gel surface. When one of the components of the solvent mixture is preferentially adsorbed by the polymer, the volume fraction of component i, using equation (12) is given by

$$\phi_i^{\lambda} = \phi_i \pm (1-\phi_3)\,\Delta\phi_{i0} \tag{18}$$

where ϕ_i is the value obtained from equation (17). The values of λ for each PS molecular weight and mixture composition for Bz/M/PS and CTC/M/PS systems have been given elsewhere[25].

The interaction parameters and their dependences on system composition for all TPS are collected in *Table 1*.

Table 2 Magnitudes needed for K_{p} evaluation from equation (6) and predicted K_{p} and K_{p}^{λ}, with and without preferential solvation, respectively, for the Bz/M/PS system at $\phi_{20} = 0.25$

Pore shape	$\bar{r}/r$	M_3[e]	υ_2	K_{p}[f]	K_{p}^{λ}[f]
sphere	2[a]	4 100	0.0095	0.86	0.86
		8 150		0.72	0.70
		17 500		0.44	0.39
		49 300		0.19	0.19
		105 000		0.03	0.03
sphere	3[b]		0.0028	0.96	0.96
				0.91	0.90
				0.76	0.71
				0.62	0.62
				0.36	0.36
sphere	2.36[c]		0.0058	0.91	0.91
				0.82	0.80
				0.59	0.53
				0.37	0.37
				0.12	0.12
sphere	3.73[d]		0.0015	0.98	0.98
				0.95	0.95
				0.86	0.83
				0.78	0.78
				0.58	0.58
cylinder	2[a]		0.0143	0.80	0.79
				0.61	0.60
				0.31	0.27
				0.08	0.08
				0.01	0.01
cylinder	3[b]		0.0056	0.91	0.91
				0.82	0.81
				0.59	0.54
				0.38	0.38
				0.12	0.12
cylinder	2.36[c]		0.0097	0.86	0.85
				0.72	0.70
				0.43	0.38
				0.18	0.18
				0.03	0.03
cylinder	3.73[d]		0.0035	0.95	0.95
				0.88	0.88
				0.71	0.66
				0.55	0.55
				0.28	0.28

[a,b] From ref. 13

[c] $r = r^{**}$, from ref. 13 (table 3)

[d] $r = r^{*}$, from ref. 13 (table 3)

[e] For $M_3 < 49\,000$ and $\phi_3' = 2.77 \times 10^{-3}$; for $M_3 > 49\,000$ and $\phi_3' = 10^{-15}$

[f] Values of ϕ_1', $\phi_1'^{\lambda}$ and ϕ_1^{λ} from equations (11), (12), (17) and (18), respectively; and the values of V_{p} for spheres and cylinders calculated with equations (13) and (14), respectively

In conclusion, all the magnitudes, with the exception of ϕ_3, in the first and second members of equation (6) can be found (see above). In *Tables 2–5* the calculated values of distribution coefficients with K_{p}^{λ} and without preferential sorption, K_{p}, are given. As can be seen in *Table 2*, the values of υ_2, and consequently of K_{p} and K_{p}^{λ} for a determined molecular weight are quite diverse. This is due to the influence of two different pore shapes considered (equations 13 and 14) and the four diverse values of $\bar{r}/r$ available in the literature. Moreover, it is also possible to observe that for the same set of values of ϕ_{20} and υ_2 the higher the molecular weight the lower the K_{p} value. This decrease implies that the higher molecular weights can display larger deviations, with respect to the equivalent hydrodynamic volume from universal calibration without the effects of secondary mechanisms ($K_{\mathrm{p}} = 1$), than the lower ones. These results have been experimentally confirmed with similar eluents and gel to ones used in this work[33]. Likewise, *Tables 2–5* show the slight influence of λ on K_{p} values. This phenomenon has been used to justify the apparent large deviations with respect to the universal calibration that polymers with lower molecular weights display in single solvents[13]. Effectively, from *Tables 2–5* it is easy to observe that for the polymers with lower molecular weights (with large λ) K_{p}^{λ} decreases slightly for the same sample and υ_2, and that for the same molecular weight and υ_2 the higher ϕ_{20} the lower K_{p}. These theoretical results are in good agreement with the experimental results[11,12]. The systems like Bz/M/PS show V_{R} values lower than the equivalent hydrodynamic volume for a reference calibration system PS–pure solvent. Therefore, from equation (10) in these cases $(V_{\mathrm{R}} - V_0)$ and K_{p} have to

Table 3 Magnitudes needed for K_p evaluation from equation (6) and predicted K_p and K_p^{λ}, with and without preferential solvation, respectively, for the Bz/M/PS system at $\phi_{20}=0.16$

Pore shape	$\bar{r}/r$	M_3[c]	v_2	K_p[d]	$K_p^{\lambda d}$
sphere	2.36[a]	4 100	0.0061	0.91	0.91
		8 150		0.82	0.81
		17 500		0.60	0.58
		49 300		0.34	0.34
		105 000		0.10	0.10
sphere	3.73[b]		0.0015	0.98	0.98
				0.95	0.95
				0.87	0.86
				0.76	0.76
				0.56	0.56
cylinder	2.36[a]		0.0102	0.85	0.85
				0.71	0.70
				0.44	0.42
				0.16	0.16
				0.02	0.02
cylinder	3.73[b]		0.0036	0.95	0.94
				0.81	0.88
				0.73	0.71
				0.52	0.52
				0.25	0.25

[a] $r=r^{**}$, from ref. 13 (table 3)
[b] $r=r^{*}$ from ref. 13 (table 3)
[c] For $M_3<49\,000$ and $\phi'_3=2.77\times10^{-3}$; for $M_3>49\,000$ and $\phi'_3=10^{-15}$
[d] Values of ϕ'_1, ϕ'^{λ}_1 and ϕ^{λ}_1 from equations (11), (12), (17) and (18), respectively; and the values of V_p for spheres and cylinders calculated with equations (13) and (14), respectively

Table 4 Magnitudes needed for K_p evaluation from equation (6) and predicted K_p and K_p^{λ}, with and without preferential solvation, respectively, for the Bz/M/PS system at $\phi_{20}=0.10$

Pore shape	$\bar{r}/r$	M_3[c]	v_2	$K_p=K_p^{\lambda d}$
sphere	2.36[a]	4 100	0.0063	0.91
		8 150		0.82
		17 500		0.63
		49 300		0.32
		105 000		0.09
sphere	3.73[b]		0.0016	0.98
				0.95
				0.88
				0.75
				0.54
cylinder	2.36[a]		0.0107	0.85
				0.71
				0.46
				0.15
				0.02
cylinder	3.73[b]		0.0036	0.94
				0.89
				0.75
				0.51
				0.23

For superscript definitions see footnote to *Table 3*

progressively diminish in order to hold the $(V_R-V_0)/K_p$ value in a single universal calibration. *Figures 2* and *3* show the plots of equation (10) for the Bz/M/PS system with eluent compositions: $\phi_{20}=0.25, 0.16, 0.10$ and 0.01. In these figures filled and open symbols stand for the experimental results of the plot $\log M[\eta]$ vs. (V_R-V_0) for Bz/PS (system without secondary mechanisms of separation, $K_p=1$), and for Bz/M/PS (system with secondary mechanisms in the separation process, $K_p\neq1$), respectively. Obviously, that kind of plot for the latter systems will fit the universal calibration reference system (Bz/PS) if an appropriate value of K_p is used ($K_p<1$). Likewise, in these figures dashed lines stand for plots $\log M[\eta]$ vs. $(V_R-V_0)/K_p$ for Bz/M/PS systems at different calculated K_p values (spherical and cylindrical pore shape and $\bar{r}/r=2.36$ and 3.73) for each ϕ_{20} (*Tables*

Table 5 Magnitudes needed for K_p evaluation from equation (6) and predicted K_p and K_p^{λ}, with and without preferential solvation, respectively, for the Bz/M/PS system at $\phi_{20}=0.01$

Pore shape	$\bar{r}/r$	M_3[c]	v_2	$K_p=K_p^{\lambda d}$
sphere	2.36[a]	4 100	0.0047	0.95
		8 150		0.90
		17 500		0.79
		49 300		0.49
		105 000		0.22
sphere	3.73[b]		0.0012	0.99
				0.97
				0.94
				0.84
				0.69
cylinder	2.36[a]		0.0078	0.91
				0.83
				0.67
				0.30
				0.08
cylinder	3.73[b]		0.0028	0.97
				0.94
				0.87
				0.66
				0.41

For superscript definitions see footnote to *Table 3*

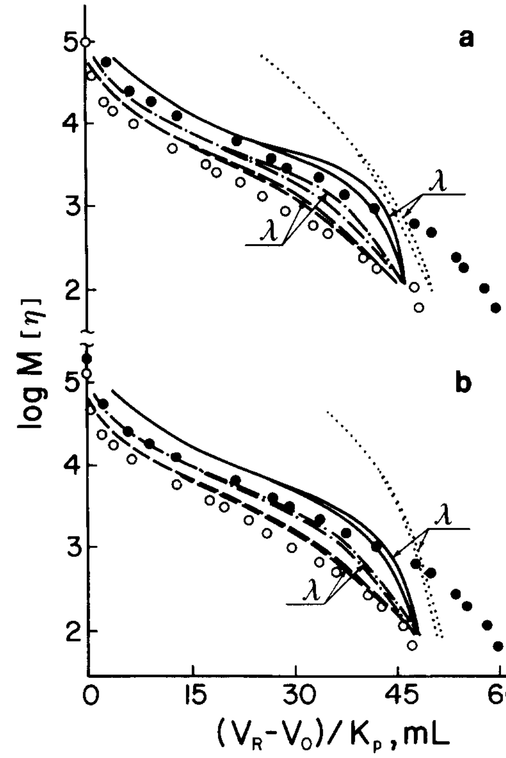

Figure 2 Universal calibration plots for benzene(Bz)/methanol(M)/polystyrene(PS) (○) and Bz/PS (●) systems at 25°C. The curves stand for theoretical calibrations obtained using the K_p values given in *Tables 2* and *3*. For spherical pore shape: $\bar{r}/r=2.36$ (——); $\bar{r}/r=3.73$ (-----). For cylindrical pore shape: $\bar{r}/r=2.36$ (······); $\bar{r}/r=3.73$ (—·—·—). The symbol λ indicates that the preferential sorption contribution has been considered. The eluent composition is: (a) $\phi_{20}=0.25$ and (b) $\phi_{20}=0.16$

2–5). For all the mixture compositions studied the best agreement between plots log $M[\eta]$ vs. $(V_R-V_0)/K_p$ and the reference universal calibration (filled points corresponding to Bz/PS system) is attained using K_p values obtained assuming a cylindrical pore shape and $\bar{r}/r=3.73$ (*Figures 2* and *3*), and especially when the preferential solvation of PS by Bz is considered, this influence being negligible for $\phi_{20}=0.10$ and 0.01. In general, the larger deviations appear in the lower polymer molecular weights. For these polymers, the theoretical results (dashed lines) would fit with the reference calibration of binary system (filled points) if K_p values were much lower than the calculated ones. This could be possible if v_2 values were much higher. However, v_2 values are independent of polymer molecular weight and fundamentally depend on the adsorption isotherm, as is clearly shown in reference 14 and in *Figure 1* in this work. It is important to point out that in those isotherms the quantity, in ml, of the polar component in the mixture adsorbed per weight unity of gel are a global average result of the adsorbed amount on pores of different sizes. In that case, a possible explanation could be that the smallest pores, with more complex geometry, would adsorb more polar component than the largest ones per weight of gel. Evidently, the pores with the smallest dimensions are exclusively accessible to the lowest molecular weights. Thus the largest deviation, with respect to the theoretical calibration curves will occur in the region of lowest M_3 when an average value of v_2 was used.

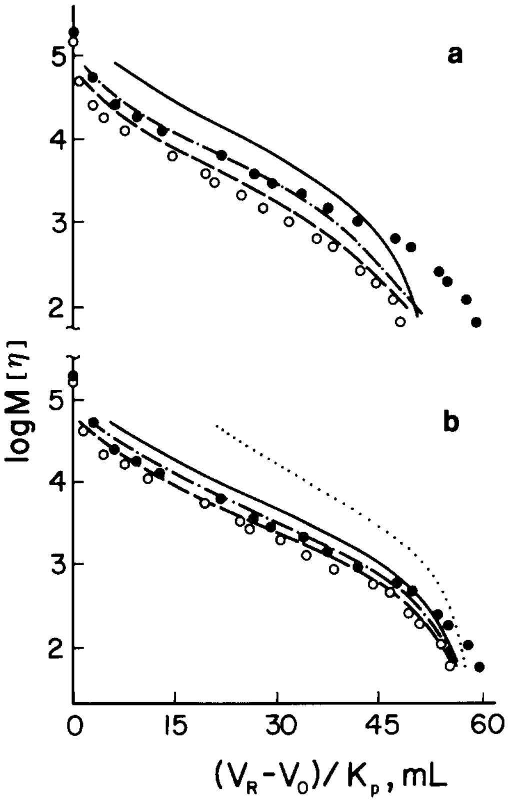

Figure 3 Universal calibration plots for Bz/M/PS (○) and Bz/PS (●) systems at 25°C. The curves stand for theoretical calibrations obtained using the K_p values given in *Tables 4* and *5*. For spherical pore shape: $\bar{r}/r=2.36$ (——); $\bar{r}/r=3.73$ (– – – – –). For cylindrical pore shape: $\bar{r}/r=2.36$ (· · · · · ·); $\bar{r}/r=3.73$ (— · — · —). The eluent composition is: (a) $\phi_{20}=0.10$ and (b) $\phi_{20}=0.01$

As has been already been mentioned for the TPS studied here, if the polymer is better solvated by the polar component, the calibration curves are shifted to lower retention volumes with respect to the reference binary system. See, for example, the TPS MEK(1)/Hep(2)/PS(3) with respect to MEK/PS. In *Table 6* values of ϕ_{10} for the MEK(1)/Hep(2)/PS(3) system and for each M_3 are detailed. Similarly for the calculations in the Bz/M/PS system, depending on the pore profile and $\bar{r}/r$ values, different v_1 are obtained, which yields diverse K_p magnitudes for the same M_3. Identical conclusions to those obtained from *Tables 2–5* could be deduced from

Table 6 Magnitudes needed for K_p evaluation from equation (6) and predicted K_p for MEK/Hep/PS system at different values of ϕ_{10}

			$\phi_{10}=0.75$		$\phi_{10}=0.60$		$\phi_{10}=0.50$		$\phi_{10}=0.40$	
Pore shape	r̄/r	M_3[c]	ϑv_1	K_p[a]	v_1	K_p[d]	v_1	K_p[d]	v_1	K_p[d]
sphere	2.36[a]	4 000	0.0030	1.02	0.0023	1.03	0.0031	1.04	0.0051	1.08
		8 150		1.05		1.06		1.10		1.21
		17 500		1.15		1.28		–		–
		49 000		1.24		1.29		1.48		2.10
		60 000		1.30		1.36		1.61		2.48
		105 000		1.59		1.71		2.30		4.90
sphere	3.73[b]		0.0015	1.01	0.0012	1.01	0.0022	1.03	0.0034	1.05
				1.02		1.03		1.07		1.13
				1.07		1.12		1.60		–
				1.12		1.14		1.33		1.63
				1.14		1.17		1.42		1.82
				1.27		1.32		1.84		2.84
cylinder	2.36[a]		0.0043	1.03	0.0034	1.04	–	–	–	–
				1.07		1.09		–		–
				1.23		1.57		–		–
				1.37		1.44		–		–
				1.47		1.57		–		–
				1.96		2.19		–		–
cylinder	3.73[b]		0.0026	1.02	0.0021	1.02	0.0026	1.03	0.0042	1.06
				1.04		1.05		1.08		1.18
				1.13		1.23		1.64		–
				1.20		1.25		1.40		1.89
				1.26		1.31		1.51		2.18
				1.49		1.60		2.05		3.95

For superscript definitions see footnote to *Table 3*

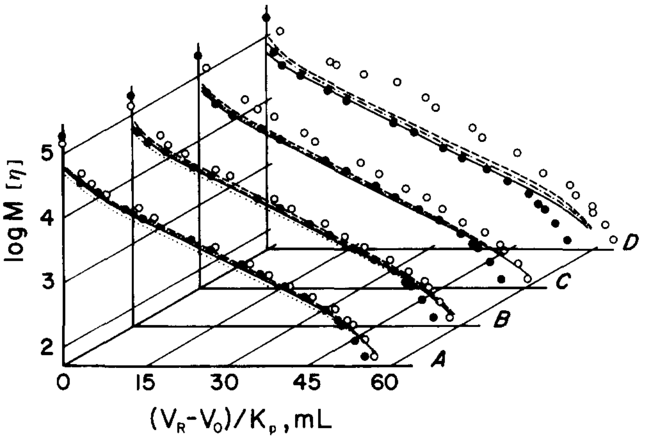

Figure 4 Universal calibration plots for butanone(MEK)/heptane-(Hep)/PS (○) and MEK/PS (●) systems at 25°C. The curves stand for theoretical calibrations obtained using the K_p values given in *Table 6* and they have the same meaning as in *Figure 3*. The eluent composition is: curve A $\phi_{10}=0.75$; curve B $\phi_{10}=0.60$; curve C $\phi_{10}=0.50$ and curve D $\phi_{10}=0.40$

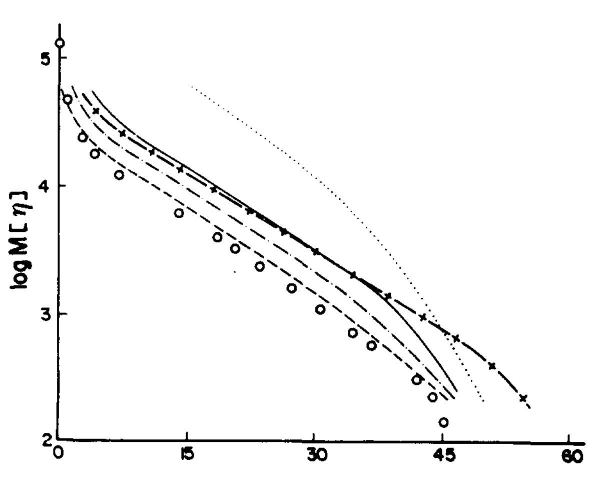

Figure 5 Universal calibration plots for carbon tetrachloride(CTC)/M/PS (○) and CTC/PS (—+—) systems at 25°C. The curves stand for theoretical calibrations obtained using K_p values (unpublished results of this laboratory) and they have the same meaning as in *Figure 3*. The eluent composition is $\phi_{20}=0.15$

Table 6, with the exception that due to the values of the interaction parameters in the MEK/Hep/PS system, $K_p>1$, as experimentally was observed[11,14]. In order to simplify the discussion, in *Table 6* all K_p values for each $\bar{r}/r$, V_p and M_3 for the MEK/Hep/PS system at different eluent compositions ($\phi_{10}=0.75$, 0.60, 0.50 and 0.40) are summarized.

In *Figure 4* the experimental results of log $M[\eta]$ vs. $(V_R-V_0)/K_p$ ($K_p=1$) for the MEK/Hep/PS system at different ϕ_{10} (empty points) and the reference calibration system MEK/PS (filled points) are shown. Likewise, in the same figure the theoretical results of log $M[\eta]$ vs. $(V_R-V_0)/K_p$ for several K_p values, calculated from equation (6), at the same ϕ_{10} (*Table 6*) are included. As is easily observed from this figure, the best fit between theoretical values of K_p and the reference universal calibration is attained when a pore with cylindrical shape and a value of $\bar{r}/r=3.73$ is considered. Analogous conclusions can be obtained for the Bz/M/PS system.

Finally, equation (6) has been also applied to cases in which the predominant secondary mechanism is the adsorption of the polymeric solute on the gel, instead of preferential solvation of the polymer by one of the components of solvent mixture that is responsible for the partition by solubility considered in this work. An example is the calibration curve log $M[\eta]$ vs. $(V_R-V_0)/K_p$ ($K_p=1$) for the system CTC/M/PS, at $\phi_{20}=0.15$, whose experimental results have been given elsewhere[13]. In *Figure 5* the theoretical results of $(V_R-V_0)/K_p$, with values of K_p calculated similarly to those for the system Bz/M/PS, are plotted for the system CTC/M/PS. The adsorption isothermal of M in the mixtures CTC-M on Spherosil given in *Figure 1* are necessary. As can be seen, some of these theoretical results agree well with ideal reference binary calibration. However, the experimental and theoretical values for the system CTC/PS cannot be compared, because for that system the PS is totally adsorbed on the gel, hence $V_R=0$ (ref. 13). The system MEK/Hep/PS shows an equivalent behaviour at $\phi_{20}<0.40$. For these mixture compositions, the polymer is completely adsorbed on the gel, therefore equation (6) cannot be used for the theoretical evaluation of K_p. Studies of thermodynamic expressions for the prediction of experimental results for the systems in which the gel is directly involved in the sorption process are in progress.

ACKNOWLEDGEMENTS

This work was partially funded by a CICYT(Spain) Grant No MAT88-0192. One of us (R.G.) was a recipient of a long-term fellowship from the Conselleria d'Educació y Ciencia (Valencia, Spain).

REFERENCES

1 Flory, P. J. and Fox, T. G. *J. Am. Chem. Soc.* 1951, **73**, 1904
2 Benoit, H., Grubisic, Z., Rempp, P., Decker, D. and Zilliox, J. G. *J. Chim. Phys.* 1966, **63**, 1507
3 Grubisic, Z., Rempp, P. and Benoit, H. *J. Polym. Sci. B* 1967, **5**, 753
4 Dawkins, J. V. and Hemming, M. *Makromol. Chem.* 1972, **155**, 75
5 Dawkins, J. V. and Hemming, M. *Makromol. Chem.* 1975, **176**, 1777
6 Iwama, M. and Homma, T. *Kogyo Kagaku Zasshi* 1971, **277**, 74
7 Dawkins, J. V. and Hemming, M. *Makromol. Chem.* 1975, **176**, 1815
8 Barrales-Rienda, J. M., Galera Gomez, P. A., Horta, A. and Saiz, E. *Macromolecules* 1985, **18**, 2572
9 Lecourtier, J., Audebert, R. and Quivoron, C. *J. Chromatogr.* 1976, **121**, 173
10 Tejero, R., Gavara, R., Gomez, C., Celda, B. and Campos, A. *Polymer* 1987, **28**, 1455
11 Berek, D., Bakos, D., Bleha, T. and Soltés, L. *Makromol. Chem.* 1975, **176**, 391
12 Bakos, D., Bleha, D., Ozima, A. and Berek, D. *J. Appl. Polym. Sci.* 1979, **23**, 2233
13 Campos, A., Soria, V. and Figueruelo, J. E. *Makromol. Chem.* 1979, **180**, 1961
14 Campos, A. and Figueruelo, J. E. *Makromol. Chem.* 1977, **178**, 3249
15 Pouchlý, J. and Zivný, A. *Makromol. Chem.* 1982, **183**, 3019
16 Pouchlý, J. and Zivný, A. *Makromol. Chem.* 1983, **184**, 2081
17 Figueruelo, J. E., Celda, B. and Campos, A. *Macromolecules* 1985, **18**, 2504
18 Figueruelo, J. E., Campos, A. and Celda, B. *Macromolecules* 1985, **18**, 2511
19 Zivný, A., Pouchlý, J. and Solč, K. *Collect. Czech. Chem. Commun.* 1967, **32**, 2753
20 Pouchlý, J., Zivný, A. and Solč, K. *J. Polym. Sci. Part C* 1968, **23**, 245
21 Martin, A. J. P. and Synge, R. L. M. *Biochem. J.* 1941, **35**, 1358

22 Dawkins, J. V. and Hemming, M. *Makromol. Chem.* 1975, **176**, 1795

23 Dawkins, J. V. *J. Polym. Sci., Polym. Phys. Edn.* 1976, **14**, 569

24 Unpublished experimental results from our laboratory

25 Soria, V., Campos, A. and Figueruelo, J. E. *Ann. Quim.* 1978, **74**, 1026

26 Mahabadi, H. K. and Rudin, A. *Polym. J.* 1979, **11**, 123

27 Campos, A., Borque, L. and Figueruelo, J. E. *J. Chromatogr.* 1977, **140**, 219

28 Donhal, V., Vins, J. and Holub, R. *Collect. Czech. Chem. Commun.* 1979, **44**, 808

29 Scatchard, G. and Ticknor, L. B. *J. Am. Chem. Soc.* 1952, **74**, 3724

30 Gavara, R., Celda, B. and Campos, A. *Eur. Polym. J.* 1986, **22**, 373

31 Masegosa, R. M., Prolongo, M. G. and Horta, A. *Macromolecules* 1986, **19**, 1478

32 Campos, A., Gavara, R., Tejero, R., Gomez, C. and Celda, B. *J. Polym. Sci., Polym. Phys. Edn.* 1989, **27**, 1569

33 Mori, S. in 'Steric Exclusion Liquid Chromatography of Polymers', (Ed. J. Janča), Marcel Dekker, New York, 1984, p. 178