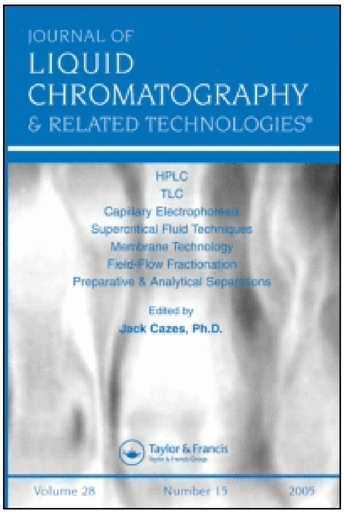

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A MODEL ACCOUNTING FOR CONCENTRATION EFFECTS IN EXCLUSION CHROMATOGRAPHY

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ABSTRACT

A model has been developed that gives a quantitative description for the dependence of the elution volume, V_e, on the concentration of injected solute, c, in exclusion chromatography (SEC). The concentration-dependent shrinkage of coils has been evaluated from the intrinsic viscosity displayed by a polymer in a binary dilute solution formed by itself (at c concentration) and the eluent. In the derived equation, concentration effects are mainly governed by the Huggins coefficient, k_H, which includes hydrodynamic as well as thermodynamic interactions. Comparisons of predicted and experimental elution volumes for diverse literature polymer/eluent/gel systems show that the model quantitatively correctly describes the dependences of concentration effects on polymer molecular weight and on thermodynamic quality of eluent.

INTRODUCTION

In exclusion chromatography (SEC) the elution volume of a macromolecular solute varies with the concentration of injected solution as it has experimentally been found both for synthetic (1-3) and for biological macromolecules (4-6). The higher the solute molecular weight and the narrower its molecular weight distribution and the better the solvent the more pronounced is the variation (7-11), a linear correlation between concentration and the elution volume at the maximum of the chromatogram, V_e, is usually found. Coil shrinkage (12-14) and viscous fingering (15,16)

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occuring with increasing concentration are claimed to be the causing agents of V_e retardation, the joint contribution of both effects being conventionally known as "concentration effects" in SEC and not included in this concept are other secondary effects as column overloading or solute sorption onto the gel (17), which also retard V_e.

SEC is conventionally used as a tool for the evaluation of molecular weight averages of polymers, through a calibration equation, usually linear, which relates the logarithms of the hydrodynamic volumes of samples with their V_e's at infinite dilution.In practice, the universal calibration curve (18) is determined by injecting solutions of standard samples at finite concentrations and although a progressive dilution of injected solutions takes place along the column, obtained V_e values are at finite concentrations and they must be corrected at infinite dilution. Many empirical correction methods (2,11,19-21) have been suggested and different theoretical models have also been proposed intending to quantitatively predict concentration effects. In Jancas's model (16,22,23) the causing agents of concentration effects are assumed to be the viscosity gradient in the zone moving along the column and the coil shrinkage, whereas in the semiempirical Rudin's model (12,24) and in Bleha's one (25) only the last effect is considered.

The concentration-dependent shrinkage of coils is usually believed to be the result of thermodynamic interactions through complicated intra- and inter-molecular excluded-volume effects (26-29). In the model proposed in this paper, it is assumed that coil shrinkage, and therefore concentration effects, are due to the joint contribution of thermodynamic and hydrodynamic interactions. Both are introduced in the model through the Huggins coefficient, k_H, on which predicted elution volumes depend.

EXPERIMENTAL

Specific viscosities at six or seven concentrations were measured with a conventional Ubbelhode viscometer. From intercepts and slopes of Huggins plots, (η_{sp}/c vs. c), Huggins coefficients, k_H were evaluated according to

$$\frac{\eta_{sp}}{c} = [\eta] + [\eta]^2 k_H c$$

The polymers used for k_H evaluations were polystyrene (PS) monodisperse samples (standard Waters Assoc.) and poly(methyl methacrylate), PMM, narrow distribution fractions ($I < 1.2$).

THEORY

A universal calibration is usually applied in SEC in order to evaluate molecular weight averages of polymers. The universal calibration concept (18) assumes a linear functionality between the log of the solute hydrodynamic volume at infinite dilution, $V_h(0)$, and solute elution volume

$$\log V_h(0) = \log M[\eta_A] = Q - PV_e(0) \qquad (1)$$

were M and $[\eta_A]$ respectively stand for molecular mass and intrinsic viscosity of a solute A and Q and P are calibration constants. $V_e(0)$ is the elution volume at infinite dilution and it cannot directly be evaluated from the chromatogram because this is run at finite concentration, c_A; of course $V_e(0) = V_{e_{c_A \to 0}}$. At a c_A concentration, coil shrinkage takes place, the hydrodynamic volume of solute A being $V_h(c_A)$ and strongly depending on c_A. Assuming that the dilution of the injected solution taking place along the column is negligible, an injected solution at c_A concentration will yield an elution volume, $V_e(c_A)$, related with $V_h(c_A)$ through eq. (1):

$$\log V_h(c_A) = Q - PV_e(c_A) \qquad (2)$$

The polymer A in a bynary mixture formed by solvent and by itself at a concentration c_A will display an intrinsic viscosity $[\eta_A]_{c_A}$ different to that in the pure solvent. Assuming that $V_h(c_A) = M[\eta_A]_{c_A}$, eq. (2) may be written as

$$\log M[\eta_A]_{c_A} = Q - PV_e(c_A) \qquad (3)$$

Subtraction of eqs. (1) and (3) yields:

$$- P(V_e(c_A) - V_e(0)) = \log\,([\eta_A]_{c_A} / [\eta_A]) \qquad (4)$$

On the other hand, the intrinsic viscosity ($[\eta_A]_{c_B}$) of a polymer A in a binary dilute solution formed by a polymer B (at concentration c_B) in a solvent is given by (30-32)

$$[\eta_A]_{c_B} = \frac{[\eta_A] + c_B b_{AB}}{1 + c_B[\eta_B] + c_B^2 b_B} \qquad (5)$$

where $[\eta_A]$ and $[\eta_B]$ are the respective intrinsic viscosities of both polymers in the pure solvent and the coefficients b_A, b_B, b_{AB} characterize the interactions of like (AA, BB) and unlike (AB) molecules, respectively.

In the present study, we are not interested on the viscometric behaviour of a polymer in a solution of a different polymer but on the viscosity of polymer A in a solution of itself at c_A concentration. In this case, eq. (5) is transformed on:

$$[\eta_A]_{c_A} = \frac{[\eta_A] + 2\,c_A b_A}{1 + c_A[\eta_A] + c_A^2 b_A} \qquad (6)$$

Eq. (6) holds for dilute solutions, that is, for low c_A values. Neglecting higher terms than c_A^2 in the expansion McLaurin's serie of the above eq., dividing both members by $[\eta_A]$, rearranging terms in c_A powers, and applying log, this is obtained:

$$\log \frac{[\eta_A]_{c_A}}{[\eta_A]} = \log\left\{1 + \frac{2b_A - [\eta_A]^2}{[\eta_A]}\,c_A + \frac{[\eta_A]^3 - 3b_A[\eta_A]}{[\eta_A]}\,c_A^2\right\} \qquad (7)$$

Changing log in the second member to ln and recalling that $\lim_{x\to 0} \ln\,(1 + x) \simeq x$ and that the interaction coefficient b_A is related to the Huggins coefficient (k_H) through $b_A = k_H[\eta_A]^2$ (31,32), eq. (7) is transformed on:

$$\log \frac{[\eta_A]_{c_A}}{[\eta_A]} = 0.4343\,\ln\left\{1+(2k_H-1)\,[\eta_A]c_A+(1-3k_H)\,[\eta_A]^2 c_A^2\right\} =$$

$$= 0.4343 \left\{(2k_H-1)[\eta_A]c_A + (1-3k_H)[\eta_A]^2c_A^2\right\} \qquad (8)$$

Finally, substitution of eq. (8) in eq. (4) yields, removing subscripts

$$V_e(c) = V_e(0) + \frac{0.4343}{P}\left\{(1-2k_H)[\eta]c + (3k_H-1)[\eta]^2c^2\right\} \qquad (9)$$

Eq. (9) shows that concentration effects in SEC for a solute depends on its intrinsic viscosity, $[\eta]$, its Huggins coefficient, k_H, and its concentration, c. Eq. (9) indirectly depends on the slope of the linear part of the universal calibration, P, defining the selectivity of the separation in a column.

RESULTS AND DISCUSSION

In good solvents and at the low injected concentrations usually used in SEC (< 5 mg/ml), the experimental evidence so far accumulated is that a linear dependence of V_e on c usually holds, steeper the slope, the higher the molecular weight of polymeric solute (7,8,10). In eq. (9) two terms account for concentration effects, one linear and the other quadratic in c. In good solvents, $k_H = 0.25 - 0.4$, therefore $|(1-2k_H)| > |(3k_H-1)|$. Moreover, in the usual measurement conditions, $[\eta]c < 1$, therefore, $[\eta]c > [\eta]^2c^2$. Both combined inequalities give the following result $(1-2k_H)[\eta]c \gg (3k_H-1)[\eta]^2c^2$, the quadratic term being negligible and a practically linear variation of V_e with c being predicted by the present model. On the other hand, when M increases, k_H decreases and both the coefficient $(1-2k_H)$ and $[\eta]$ increase; as a result, a steeper dependence on c of V_e is also predicted. In same polymer-solvent systems, at concentrations higher than $c \simeq 0.5$ mg/ml, the inicial straight lines V_e vs. c become curved either upwards or downwards depending on the polymer eluent system and both curvatures becoming steeper with increasing molecular weight. Eq. (9) also accounts for both experimental behaviours. So, when $k_H > 1/3$ rising curvatures must be expected, whereas downward ones are predicted in better solvents, $k_H < 1/3$. Of course, when $k_H=1/3$, linearity holds even at moderate concentrations. Qualitatively, then, the present model is able to account for the different concentration effects experimentally observed in good eluents.

Difficulties arise, however, when intending to compare quantitative predictions of this model with literature experimental results. On one hand, because of the scarcity of literature references, in which both elution volumes and their corresponding viscosity coefficients for the polymeric solutes are given. On the other hand, when looking for elution volumes and k_H data separately, not very accurate values may be found because:

i) Although plentiful concentration effects data may be found in the literature, most of these are shown in a graphic form, and in a single figure data for several polymer samples with very different molecular weights are usually represented. The differences in elution volumes between different polymer samples are usually several magnitude orders higher than the differences caused by concentration effects, therefore, the uncertainty in the V_e numerical values extracted from literature figures may be about the same magnitude order than the expected concentration effects.

ii) Besides that, the chances of finding appropriate k_H values for the above eluent-polymer sample-temperature systems are remote. What makes such undertaking particularly difficult is that k_H depends not only on polymer-solvent system, but also on molecular weight distribution of polymer, branching degree of the chain and velocity gradient (33). In the present context, the last two effects may be neglected. No agreement exists on the dependence of k_H on molecular weight; it seems that with low molecular weight k_H decreases as M increases to a certain M limit, beyond which k_H is practically insensitive to M (33,34). Fortunately, it seems that molecular weight distribution does not significantly influence k_H (33).

iii) Added to the above problems is the rather considerable limit of error of experimental k_H values, which can amount to 10-20 % (33).

Taking into account the above considerations, literature references on concentration effects may be grouped in three categories:

1. Those in which both numerical values for V_e (usually tables) and k_H values for the polymeric solutes are given.

2. Literature reports in which V_e's are also given in tabulated form,but k_H values for the measured polymers are not reported. In these cases, k_H values must be searched for in other literature sources. Three possibilities, in a decreasing order of preference, have been followed in this paper:

a) When existing k_H - M data, the corresponding interpolated values have been used.

b) If possibility a) does not exist, used k_H values are those found in the literature for the closest temperature and/or molecular weight polymer.

c) If even this possibility fails, k_H experimental evaluations were undertaken. The polymers used for those evaluations were monodisperse samples or narrow distribution fractions with the closest possible molecular weight to the chromatographic samples.

3. Most of the literature references on concentration effects fall into this group. V_e's are given here in figures. When existing k_H values in the same reference, those have been used; when not, the considerations above made have also been followed.

As far as we know, only one system, namely polystyrene, PS, (M_w = 867000)/tetrahydrofuran, THF, falls into group 1. It was measured by Janca to test the realibility of its own concentration effects model (16). In table 1, concentration effects predicted by Janca (ΔV_J) and by the present model (ΔV_F) are shown at different concentrations of injected solution. Concentration effects (ΔV_e) are defined as the difference between the elution volumes at

TABLE 1

Concentration Effects in the System PS3(M_w = 867000)/THF. Comparison between Experimental (16), ΔV_e, and predicted Values from Janca's Model (16), ΔV_J, and from the present Model, ΔV_F.

c g/100 ml	V_e counts	ΔV_e counts	ΔV_J counts	ΔV_F^* counts
0.4	22.8	1.3	0.2	0.9
0.2	22.3	0.8	0.02	0.4
0.1	21.8	0.3	0.00	0.2
0.05	21.7	0.2	-0.05	0.1
0.025	21.6	0.1	-0.07	0.04
0	21.50			

* Data needed to calculate ΔV_F are from ref. (16) and are in Table 3.

concentration c, $V_e(c)$, and at infinite dilution, $V_e(0)$, in eq. (9).

In Table 2 elution volumes at several concentrations as predicted by the present model are given for systems falling into group 2. The first two systems were, respectively, used by Janca (16) and by Rudin (12) as test systems of their own models. The three different behaviours theoretically predicted by our model in good solvents, that is, a practically linear, a curved upwards and a curved downwards dependence of V_e are illustrated in Figure 1, for several systems falling into group 3. Data for eq.(9) application in good solvent systems are enclosed in Table 3. Expected elution volumes from Bleha's and Rudin's models are also depicted in Figures 1b) and 1c), respectively. No significative differences, from a quantitative point of view, are found in good solvents between our predicted elution volumes and those derived from other models, as Figures 1b) and 1c) show. Of course, if the quadratic term in eq. (9) is neglected, as it does not play an important role in the systems so far represented in Figures 1b)

TABLE 2

Concentration Effects in Different Polymer/Eluent/Gel Systems. Comparison between Experimental, V_e, and Calculated with eq. (9), $V_e(c)$, Elution Volumes.

a) PS/THF/rigid porous glass at 25°C (16)

Sample	c,%	V_e, counts	$V_e(c)$,* counts	Sample	c,%	V_e, counts	$V_e(c)$,* counts
PS 4 498000	0.8	25.8	25.3(24.5)	PS 6 200000	0.4	29.2	29.1(28.9)
	0.4	25.2	24.7(24.4)		0.2	28.9	29.0(28.8)
	0.2	24.7	24.4(24.3)		0.1	28.9	28.9(28.8)
	0.1	24.4	24.2(24.2)		0.05	28.9	28.9(28.8)
	0.05	24.2	24.2(24.2)		0.025	28.9	28.9(28.8)
	0.025	24.1	24.1(24.2)		0	28.95	
	0	24.1					

b) PMMA/THF/styragel at 25°C (35)

Sample	$c.10^3$ g/ml	V_e, ml	$V_e(c)$, ml	Sample	$c.10^3$ g/ml	V_e, ml	$V_e(c)$, ml
Fract. A1 $M_w=246.10^4$	0	102.8		Fract. B2 $M_w=596.10^3$	0	114.9	
	1.13	103.5	103.5		1.64	115.5	115.3
	3.13	104.5	104.6		3.28	116.0	115.7
	6.46	106.0	106.5		6.58	117.0	116.5
	9.74	108.0	108.2		9.55	118.0	117.3
	12.7	111.0	109.8		13.15	119.5	118.4
Fract. B1 $M_w=440.10^3$	0	117.1		Fract. C2 $M_w=240.10^3$	0	123.0	
	1.59	117.5	117.4		1.61	123.0	123.2
	3.15	118.0	117.7		3.21	123.5	123.4
	6.66	119.0	118.4		6.35	124.0	123.8
	9.77	120.0	119.1		9.66	124.5	124.2
	13.2	121.0	119.9		13.15	125.0	124.7

c) PS/THF/μstyragel at 25°C (36)

Sample	$c.10^3$ g/ml	V_e, counts	$V_e(c)$, counts	Sample	$c.10^3$ g/ml	V_e, counts	$V_e(c)$, counts
PS 620.10^3	0	26.18		PS 412.10^3	0	27.04	
	1.4	26.27	26.29		1.1	27.10	27.11
	2.2	26.34	26.36		1.3	27.12	27.13
	3.4	26.43	26.48		2.1	27.15	27.17
					2.9	27.20	27.23

* Values in parenthesis are $V_e(c)$ predicted by Janca (16).

TABLE 3

Parameters used for the Evaluation of Concentration Effects in Good Solvents

System	Sample	P	$[\eta]$ ml.g^{-1}	k_H
PS/THF (16), Table 1	PS 3	0.145 counts^{-1}	212	0.365
PS/THF (16), Table 2a	PS 4	0.145 counts^{-1}	142	0.366 a
	PS 6		74	0.367 b
PMMA/THF (35), Table 2b	Fraction A1	0.079 ml^{-1}	329	0.33 c
	Fraction B2		124 (12)	0.35
	Fraction B1		100	0.36
	Fraction C2		66	0.36
PS/THF (36), Table 2c	PS 620000	0.30 counts^{-1}	185	0.366 a
	PS 412000		150	0.367
PS/THF (37), Figure 1a	1.8×10^{6} PS	0.068 ml^{-1}	422	0.325 c
	860000 PS	0.068 ml^{-1}	240	0.365(16)
	200000 PS	0.075 ml^{-1}	78	0.367 b
PS/Toluene (25), Figure 1b	$M\approx4.98\times10^{5}$	0.068 ml^{-1}	141	0.33 (33)
	$M\approx3.20\times10^{5}$		88	0.35 (33)
PS/Butanone (38), Figure 1c	$M\approx4.98\times10^{5}$	0.256 counts^{-1}	81	0.55 c
	$M\approx3.35\times10^{6}$		271	0.48

a. Interpolated values from k_H vs. M data in ref. (16).
b. Extrapolated values from k_H vs. M data in ref. (16).
c. Measured in this lab.

and 1c), the new eq. describing concentration effects (eq. (10)) obeys a similar functionality to those derived from Bleha's model (eq. (11)) and Rudin's model (eq. (12)) :

$$V_e(c) = V_e(0) + \frac{0.4343}{P}(1-2k_H)[\eta]c \qquad (10)$$

$$V_e(c) = V_e(0) + \frac{1.303}{P} A_2 \; M \; F \; c \qquad (11)$$

$$V_e(c) = V_e(0) + \frac{0.352}{P} (1 - [\eta]_\theta/[\eta])[\eta]c \qquad (12)$$

Eq. (12) proceeds from a rearrangement of the original Rudin's eq. (see eq. (24) in ref. (24)).

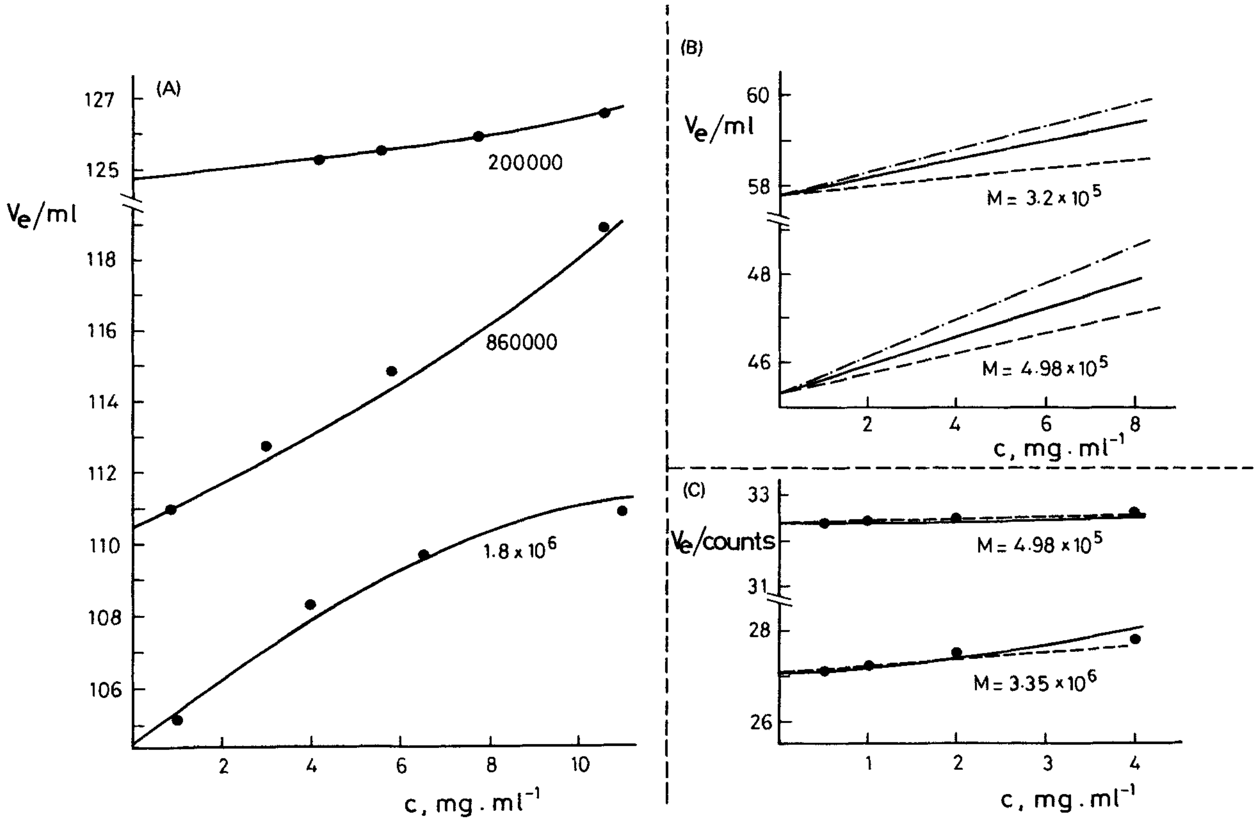

FIGURE 1. Calculated and experimental concentration effects for the systems: a) PS/THF; (●) experimental values (37); (———) predicted with eq.(9). b) PS/toluene; (—·—·) experimental values (25); (———) predicted with eq.(9); (----) predicted with Bleha's model (25). c) PS/butanone; (●) experimental values (38); (———) predicted with eq.(9); (----) predicted with Rudin's model.

The small differences, among the three models, then, must be looked for in the coefficient of the variable c/P and those differences may be caused mainly by the different experimental magnitudes on which the coefficients depend on. Bleha's model(25) is based upon Yamakawa's theory (29), whereas the late Rudin's model (24), even though it was firstly derived in a semiempirical way (12), also agrees with Yamakawa theory relating concentration and effective hydrodynamic volume of solvated polymers in moderately concentrated solutions. Therefore, both models are two different ways of presenting Yamakawa theory, in Bleha's model elution volumes are given in terms of equilibrium properties ($A_2 \equiv$ second virial coefficient) wherein Rudin's model they are a function of transport properties ($[\eta] \equiv$ intrinsic viscosity). However and in spite of the almost quantitative agreement found between our model and those based on Yamakawa theory, a qualitative disagreement, intrinsic in nature, exists.So, wherein the models based in Yamakawa theory the concentration-dependent shrinkage of coils is a result of thermodynamic interactions including inter- and intramolecular excluded-volume effects, our model depends on k_H, which certainly includes thermodynamic interactions, but also includes hydrodynamic and other types of interactions, being the most important both thermodynamic and hydrodynamic interactions (31-33). This conceptual difference is not largely reflected on the predicted elution volumes in good solvents, as previously shown, but it is however at θ conditions. In fact, at θ conditions thermodynamic interactions vanish, the ratio $[\eta]/[\eta]_\theta$ and the expansion factor being equal to unity and the function F being zero. As a result, see eqs. (11) and (12), a universal behaviour is predicted by both Bleha's and Rudin's model: elution volumes do not depend on concentration at θ conditions, which is not the behaviour predicted by our model. Elution volumes in our model depend on k_H and since not a single, therefore universal, k_H value is found at θ conditions, either theoretical or experimental, not an universal concentration dependence of V_e must be expected. Among other theoretical predictions, Yamakawa arrives at $(k_H)_\theta = 0.50$, taking only into account the effect of the concentration dependence of the molecular dimensions (39). Peterson and Fixman predict that $(k_H)_\theta = 0.833$ facing the hydrodynamic problem (40) and a value $(k_H)_\theta = 0.52$ is claimed by Sakai (41). On the other hand, experiments show that $(k_H)_\theta$ lies between 0.5 and 0.7 (31,42,43) and it is molecular weight dependent. In this context, it is obvious that not a universal behaviour of elution volumes as concentration independent must be expected in θ eluents.

Before presenting quantitative results in θ eluents, a qualitative analysis of the results predicted by our model may be worthwhile . At an hypothetical $(k_H)_\theta = 0.50$, in accordance with the Yamakawa's theoretical $(k_H)_\theta$ value, the linear term in eq.(9) vanishes as it also happens with thermodynamic interactions according to Yamakawa (39), but the quadratic term still remains. In this context, the quadratic term looks like accounting for hydrodynamic and other kinds of interactions, which are responsible of the residual concentration dependence of elution volumes at those θ conditions. Of course, $(3k_H-1)$ coefficient is larger here than in good solvents, whereas $[\eta]_\theta$ is smaller than $[\eta]$. The increase in the former is cancelled by the decrease in the latter and as a result the value of the quadratic term is small and about in the same order as in good solvents. A small positive V_e vs. c trend must, then, be expected in $(k_H)_\theta = 0.50$ systems. For those systems, with $(k_H)_\theta$ values close to the limiting 0.50, the linear coefficient $(1-2k_H)$ becomes negative and, of course, much smaller than the quadratic coefficient, $|(1-2k_H)| << |(3k_H-1)|$. However, here again, as in good solvents, $[\eta]_\theta c > [\eta]_\theta^2 c^2$, and the linear term provokes a decrease in the small predicted dependence of V_e with c followed at $(k_H)_\theta$ 0.50. As long as $(k_H)_\theta$ departs farther from 0.50, the differences between linear and quadratic terms become smaller, the elution volumes depending less on c and that dependence vanishing at a limit $(k_H)_\theta$ value and even becoming negative at low concentrations and for $(k_H)_\theta$ values above the limit. On the other hand, below a given molecular weight, $(k_H)_\theta$ strongly increases with decreasing molecular weight (42,34), high $(k_H)_\theta$ values being reached for low molecular weight polymers and a negative dependence of elution volumes with c must, then, be expected. However, in these low molecular weight polymer systems, $[\eta]_\theta$ is small and the expected negative dependence, if any, will be negligible. In any case, and as a summary, at θ conditions and for high molecular weight polymers a small positive, if any, V_e dependence on c must be expected, which will gradually diminish as long as the polymer molecular weight decreases and for very low molecular weight polymers even can become negative.

The difficulties appearing when intending to compare our quantitative predictions for θ systems with literature experimental results are enhanced with respect to those found and discussed before for good solvent systems. Here, tabulated V_e vs. c were not found and we must retort upon data of group 3. Besides that, and because of the small differences in elution volumes usually found, close to the accuracy in the V_e experimental measurements, horizontal straight lines in the plots V_e vs. c are usually depicted, masking the experimental points and therefore spreading the generalized opinion of the V_e independence with c at θ conditions. The uncertainty in numerical values extracted from literature figures here may be higher than expected concentration effects.

The three different behaviours theoretically predicted by our model in θ conditions, that is, a positive, a null and a negative concentration dependence of V_e, as long as $(k_H)_\theta$ increases, are illustrated in Figure 2, where predicted and experimental V_e vs. c plots for polystyrene (PS) in different θ mixtures are compared. For $(k_H)_\theta$ close to 0.50, as it occurs in the benzene/methanol (78/22, v/v) θ mixture, a small positive concentration dependence of V_e is experimentally observed (7), as explicitly confirmed by authors, and in accordance with

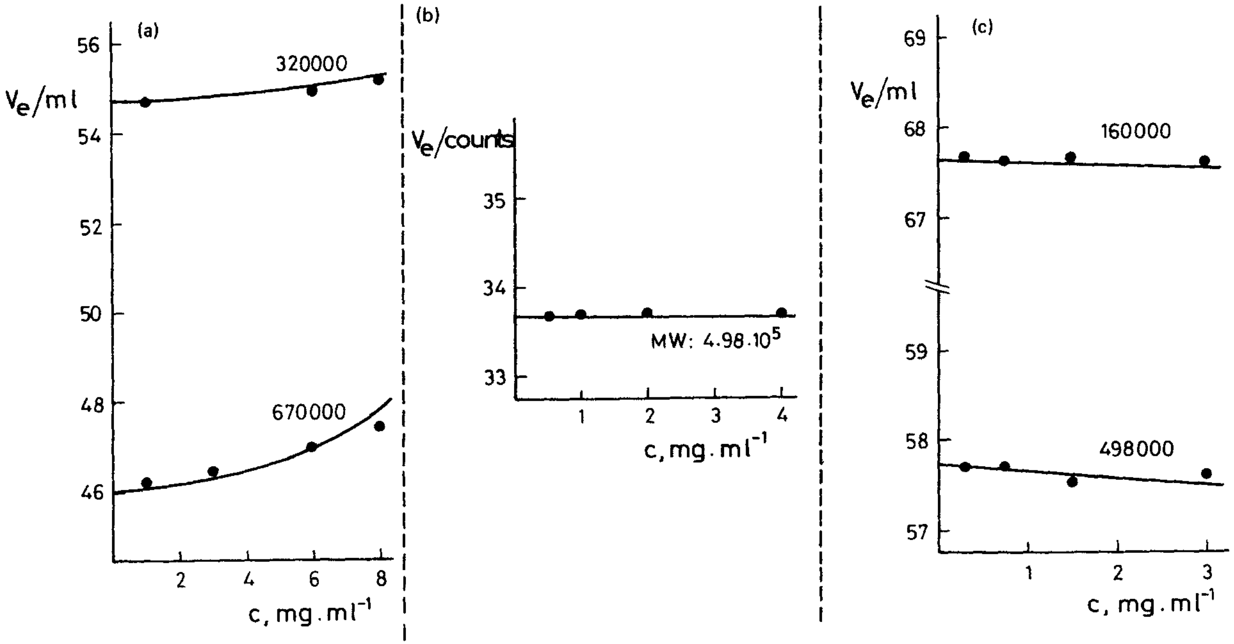

FIGURE 2. Calculated and experimental concentration effects for PS in θ eluents; (●) experimental values; (——) predicted with eq.(9). a) benzene-methanol(78/22) (7). b) butanone-methanol (88.7/11.3) (19). c) butanone-n,heptane(50/50) (44).

TABLE 4

Parameters used for the Evaluation of Concentration Effects in θ Eluents.

System	Sample	P	[η] ml.g^{-1}	k_H
PS/benzene-methanol (78/22),v/v) (7), Figure 2a.	670000 320000	0.069 ml^{-1}	93 60	0.50 a 0.51
PS/butanone-methanol (88.7/11.3,v/v) (19) Figure 2b.	4.98×10^{5}	0.261 counts^{-1}	51 a	0.63 a
PS/butanone-n,heptane (50/50,v/v) (43), Figure 2c.	498000 160000	0.074ml^{-1}	59.5 a 33.7	0.73 a 0.70

a. Measured in this lab.

theoretical predictions. For intermediate $(k_H)_\theta$ values a null dependence must be expected, as it is experimentally confirmed in the θ mixture butanone/methanol (88.7/11.3, v/v) (19) and for high $(k_H)_\theta$ values a very small negative dependence must be obeyed, as also happens in the mixture butanone/n-heptane (50/50, v/v)(43). Data for eq. (9) application in θ eluents are enclosed in Table 4.

Finally and as a conclusion, the applicability of the present model deserves some comments. An appropriate calibration in SEC demands [η] values, but the evaluation of [η]'s yields at once the corresponding k_H values. No additional data, then, is needed to calculate concentration effects, since these only depend on [η] and k_H.

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