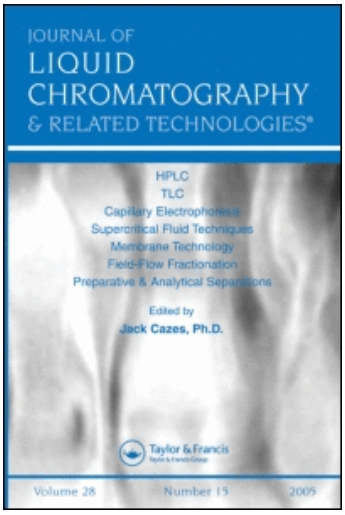

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On the Concentration Effects in Steric Exclusion Chromatography Under Stationary Equilibrium Conditions

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ON THE CONCENTRATION EFFECTS IN STERIC EXCLUSION CHROMATOGRAPHY UNDER STATIONARY EQUILIBRIUM CONDITIONS

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ABSTRACT

The effect of concentration on the distribution of macromolecules between solution inside the pores and outside the porous medium after mixing of polymer solution with the medium was investigated. Experimental measurements were carried out for polystyrene standards in a thermodynamically good solvent - tetrahydrofuran, and in a mixed theta solvent tetrahydrofuran-methanol. The results of measurements, particularly in the theta solvent, suggested a considerable effect of secondary non-exclusion interactions. A comparison between the distribution coefficients calculated theoretically using various models and those determined experimentally revealed a considerable discrepancy. It is obvious that the reported theoretical models of concentration dependence of the distribution coefficients under stationary conditions do not adequately reflect the real situation. The individual likely causes of this discrepancy have been critically discussed.

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INTRODUCTION

In our preceding paper (1) we reported an experimental investigation of the concentration dependence of the equilibrium distribution coefficient K_{SEC}. The measurements were carried out under stationary conditions, by mixing a known quantity of the porous medium with a known volume of polymer solution of the given concentration. After the equilibrium was established the polymer concentration in the supernatant was determined. If the dissolved polymer was excluded at least from one part of the pores accessible to pure solvent, the polymer concentration in the supernatant increased. Using the values of polymer concentration in solution before mixing with the porous medium and after mixing the known volume of the solution and the known pore volume in the porous medium, the equilibrium distribution coefficient can be calculated. Its dependence on the polymer concentration in solution is interesting, because values thus determined are free from the effect of dynamic factors, such as, e.g., viscosity phenomena operative to a considerable degree in concentration effects which have been studied by us and quantitatively described in a series of our preceding papers (for review cf. (2)). The results of our preceding paper (1) showed that the experimentally determined increase in the distribution coefficient with increasing concentration is larger than corresponds to the theoretical calculations based on the assumed role played merely by a change in the effective size of dissolved macromolecules with varying concentration. This has proved that the contribution of secondary exclusion, the role of which had been considered earlier, may be only very small, if any. If secondary exclusion was also operative in the concentration effects, this would lead, on the

contrary, to a decrease in the distribution coefficient with increasing polymer concentration in solution, or a smaller increase in the distribution coefficient with increasing concentration than corresponds to a change in the effective size of macromolecules.

Anderson and Brannon (3) reported a theoretical model of concentration dependence of the distribution coefficient of rigid spherical macromolecules in the porous structure. Their model is interesting, since it predicts concentration effects also in the thermodynamically poor theta solvent.

This study supplements our preceding measurements (1) and extends experimental measurements of the equilibrium distribution coefficients K_{SEC} under stationary conditions, also using the theta solvent. By comparing all experimental results thus obtained with the theoretically calculated distribution coefficients, various theoretical models were critically analyzed which, in principle, may elucidate the observed concentration dependence of the distribution coefficients.

THEORY

No detailed analysis of the individual theoretical models of concentration effects is offered in this part. Only basic theoretical relations are given which are used in the interpretation of the experimental results and which make possible a basic understanding of the problem of concentration effects. For details, we refer to our earlier papers.

For the concentration ratio of the polymer solution before mixing with the porous medium, c_1, and after the mixing, c_2, relation

$$c_1/c_2 = 1 + (V_p/V_1)(K_{SEC}-1) \quad (1)$$

is valid, in which V_p is the total pore volume in the porous medium added, V_1 is the volume of polymer solution before mixing, and K_{SEC} is the distribution coefficient (1). It is assumed that for infinite dilution the equality between the distribution coefficients under stationary conditions holds also in a dynamic chromatographic experiment. The distribution coefficient under dynamic chromatographic conditions is defined by

$$V_R = V_i + K_{SEC}\, V_p \quad (2)$$

where V_R is the retention volume of the given polymer and V_i is the interstitial or dead volume of the solvent between grains of the column packing. For the totally excluded polymer, $K_{SEC} = 0$. If there is no other interaction between the separated macromolecules and porous medium apart from steric exclusion, for permeating macromolecules $0 \leq K_{SEC} \leq 1$. The dependence of the distribution coefficient on the effective dimensions of permeating macromolecules may be described by an empirical calibration function

$$K_{SEC} = f\,(v.\varepsilon) \quad (3)$$

where v is the volume of unswollen macromolecule and ε is the dimensionless swelling factor. The central part of the calibration curve may be approximated by a linear function

$$K_{SEC} = P + Q \ln (v.\varepsilon) \quad (4)$$

According to Rudin and Wagner (4), the swelling factor

ε is a function of the concentration c

$$1/\varepsilon = 1/\varepsilon_o + (c/c_x)(\varepsilon_o - 1)/\varepsilon_o \quad (5)$$

where ε_o is the swelling factor for infinite dilution (c=0) and c_x is the critical concentration at which the size of the macromolecule is the same as under the theta conditions (ε=1). These quantities can be calculated from

$$\varepsilon_o = [\eta]/[\eta]_\theta \quad (6)$$

$$c_x = M/(N_o\ v) \quad (7)$$

$$v = 4\pi[\eta]_\theta M/(3.\Phi) \quad (8)$$

where $[\eta]$ is the intrinsic viscosity; in the theta solvent, $[\eta]_\theta$ is given by

$$[\eta]_\theta = K_\theta \cdot M^{0.5} \quad (9)$$

N_o is the Avogadro number, M is molecular weight and $\Phi = 3.1 \times 10^{24}$ is the universal Flory constant.

Anderson and Brannon (3) have suggested a model which describes the distribution of rigid spherical macromolecules between solution in pores of various shape, on the one hand, and bulk solution, on the other, assuming steric (hard sphere - hard wall) and long-range (screened electrostatic) interactions. These authors described, in terms of virial series expansion, the concentration dependence of the distribution coefficient for the single particle distribution function in a restricting medium, where the effect of the porous medium is represented by position-dependent potential energy acting upon each macromolecule. They explained the concentration effect

on the distribution function by coupling between the macromolecule-macromolecule and macromolecule-pore interactions. The basic idea underlying the effect of concentration on the local distribution coefficient consists in a reduced spherical symmetry inside the pore which causes a decrease in the macromolecule--macromolecule interaction inside the pore. As a consequence, the same interactions outside the pores enhance the distribution of macromolecules towards solution in the pore, compared with the exclusion effect of the single macromolecule-pore wall interaction. The result of complicated operations involving mathematical statistics was approximated (3) by means of an empirical virial expansion

$$K_{SEC} = K_o \ (1 + \alpha_1 \ c_\infty + \alpha_2 \ c_\infty^2 + \ ...) \qquad (10)$$

where K_o is the distribution coefficient at zero concentration and c_∞ is the polymer concentration in the supernatant. Hence, in the stationary experiment, $c_2 = c_\infty$. The first virial coefficient α_1 was then given by

$$\frac{\alpha_1}{v} = 8 - 7.92 \ K_o - 8.48 \ K_o^2 + 8.40 \ K_o^3 \qquad (11)$$

The preceding theoretical conclusions offer two limiting hypothetical alternatives for the explanation of the concentration effect under stationary conditions. First, it may be assumed that a change in the distribution coefficient is due only to a change in the effective dimensions of the macromolecule with changing concentration. In this case, however, the distribution coefficient should not be affected by concentration in the theta solvent. In the second case of the Anderson-Brannon model (3), the distribu-

tion coefficient ought to be a function of concentration also for rigid macromolecules; hence, it should vary also in the theta solvent, in spite of the fact that there is no change in the effective size of macromolecules with varying concentration. Our experimental work was planned so as to make possible a critical comparison of the alternatives just outlined with real experimental data.

EXPERIMENTAL

Polystyrene standards (PS) were used; their molecular parameters are given in Table 1. Tetrahydrofuran (THF) representing a thermodynamically good solvent and a mixture of THF and 28.7 % (v/v) methanol which at 25 °C is a theta solvent (5) were used as solvents. A detailed description of experimental procedures has been given in our preceding paper (1), from which also one part of experimental results has been taken. In this study, a constant temperature of 25 °C (accuracy ± 0.1 °C) was maintained in all experiments by means of a thermostat. The porous medium (silicagel Merckogel Si 500 Å deactivated by a reaction with trimethyl chlorosilane and hexamethyl dichlorosilane, particle size 40-63 μm) was mixed with a solution of PS after evacuation of the mixing vessel containing the porous medium. This eliminated the possibility of air microbubbles being caught in the pores. The ratio of concentrations of PS solutions before and after mixing with the porous medium was determined chromatographically as reported earlier (1).

Porosity (the total pore volume of the given amount of porous medium) was determined by the method of

TABLE 1

Molecular parameters of polystyrene standards

Standard	Manufacturer	$M_w x10^{-3}$	$M_n x10^{-3}$
PS 694 000	Waters Assoc.,Inc.	694	not given $M_w/M_n < 1.05$
PS 303 000	Chrompack,Holland	303	288
PS 111 000	Waters	111	111

Note: Molecular weights are given by the manufacturer

mercury porosimetry. The total pore volume of Merckogel Si 500 Å was V_p = 0.641 ml/g.

The composition of the theta solvent before and after mixing with Merckogel Si 500 Å was determined by gas chromatography. 0.2275 g of silicagel was mixed with 0.4677 g of the theta solvent. The methanol content in the supernatant dropped from the original 28.7 % v/v to 27.2 % v/v. This fact indicates that under the given conditions, methanol is probably preferentially sorbed on the surface of the porous medium, and consequently the composition of the solvent varies near the surface. For this reason, a one-component theta solvent seems more suitable. On the other hand, however, the unpublished results of our preceding paper (1) demonstrated a considerable adsorption of PS on the surface of the porous medium, if cyclohexane was used as the theta solvent. A similar adsorption was observed with deactivated Merckogel Si 500 Å. Virtually, the adsorption was so strong that the polymer concentration in the supernatant decreased distinctly, so that, at low concentrations,

the polymer could not be detected in the supernatant at all. It is of interest that, although in the stationary arrangement the polymer was almost quantitatively adsorbed from the solution in cyclohexane in the dynamic chromatographic experiment with cyclohexane as solvent the experimental result did not suggest adsorption.

RESULTS AND DISCUSSION

In addition to direct experimental data obtained by the stationary measurement, the Mark-Houwink equation for PS in THF at 25 °C was also needed for the calculations. We used an equation from our earlier work (6)

$$[\eta] = 1.17 \times 10^{-2} M^{0.717} \qquad (12)$$

The constant of Eq. (9) was calculated as $K_\theta = 8.12 \times 10^{-2}$ (1). The slope and abscissa of the calibration function (Eq. (4)) were calculated for Merckogel Si 500 Å by using the least squares method from chromatographic data obtained by the calibration of the PS series by employing a procedure described earlier (1). The values thus obtained were $P = -4.5178$ and $Q = -0.1201$.

The experimental c_1/c_2 and V_p/V_1 values are reviewed in the first part of Table 2. The second part of Table 2 contains the distribution coefficients K_{SEC} calculated using Eq. (1) from direct experimental data (A) in THF (a,b) and in the theta solvent (c). Also, the Table 2 contains the K_{SEC} values calculated theoretically assuming that a change in concentration will affect the change in K_{SEC} only

TABLE 2

Change in the distribution coefficients K_{SEC} due to a change in concentration in the stationary experiment - direct experimental values and theoretical calculation according to various models

c_1 (% w/v)	c_1/c_2	V_p/V_1	K_{SEC} A	K_{SEC} B	K_{SEC} C
PS 694 000[a]					
4.21	0.9109	0.286	0.689	0.273	0.612
3.11	0.8802	0.281	0.574	0.246	0.581
1.41	0.8390	0.267	0.396	0.179	0.473
1.13	0.8377	0.241	0.327	0.162	0.436
0.52	0.7359	0.324	0.185	0.122	0.327
0.125	0.7023	0.325	0.084	0.077	0.153
0				0.057	0.057
PS 303 000[b]					
1.87	0.9611	0.159	0.755	0,085	0.100
0.88	0.9052	0.133	0.288	0.053	0.070
0.43	0.8395	0.207	0.225	0.034	0.048
0.21	0.8286	0.187	0.083	0.024	0.032
0				0.013	0.013
PS 303 000[c]					
1.85	0.8917	0.204	0.469	0.013	0.099
0.92	0.8596	0.204	0.312	0.013	0.078
0.45	0.8516	0.191	0.223	0.013	0.050
0.22	0.8087	0.198	0.032	0.013	0.033
0				0.013	0.013
PS 110 000[a]					
2.00	0.9642	0.216	0.834	0.509	1.392
1.00	0.9330	0.240	0.721	0.492	1.016
0.50	0.8885	0.259	0.570	0.482	0.779
0.13	0.8765	0.278	0.556	0.473	0.553
0.03	0.8834	0.225	0.483	0.470	0.490
ↄ			0.469	0.469	

Note:

A - calculated from experimental data using Eq.(1)

B - calculated theoretically: only the effect of a change in the effective dimensions of macromolecules; Eq.(4).

C - calculated theoretically: only the effect of macromolecule-macromolecule interactions; Eqs. (10) and (11).

a - experimental values from ref. (1) in tetrahydrofuran

b - stationary experiment in tetrahydrofuran

c - stationary experiment in theta solvent

as a consequence of the change in effective dimensions of macromolecules in solution. Using the dynamic chromatographic experiment, the empirical calibration function, i.e. the dependence of K_{SEC} on the effective dimensions of PS of various molecular weights, and the P and Q values were determined. The effective dimensions of PS macromolecules for various concentrations were calculated from Eqs. (5-9). Finally, the distribution coefficients were calculated from Eqs. (10) and (11). From the results summarized in Table 2, several conclusions may be drawn:

1. K_{SEC} calculated from experimental data for PS 303 000 varies in an important way also in the theta solvent. As K_{SEC} of the same PS in THF changes much more with changing concentration than values for PS 694 000, is seems that also interactions other than mere steric exclusion take place on the deactivated Merckogel Si 500 Å. Measurements with PS 694 000 and PS 111 000 obtained earlier (1) were performed with Porasil DX, but other interactions also cannot be excluded even in this last case.

2. The K_{SEC} values calculated only from a change in the effective dimensions of macromolecules in solution are distinctly lower in all cases than corresponds to experimental ones. This finding has been reported earlier (1). It is quite obvious, therefore, that a mere change in effective dimensions with changing concentration cannot explain the overall observed change in K_{SEC}, even under conditions of a stationary experiment. Already from these facts it can be seen that, as long as one does not succeed in excluding or quantitatively describing some interactions other than mere steric exclusion, the stationary experiment has only a limited

importance in the investigation of concentration effects in SEC, and also in corroborating any model of the separation mechanism in SEC (7).

3. The K_{SEC} values calculated using the Anderson and Brannon model suggest an apparent accord with the experimental values for PS 694 000. For PS 303 000 they are substantially lower than the experimental ones. For PS 111 000, on the other hand, they are much higher, reaching even extreme and physically unlikely values $K_{SEC} > 1$. Only the first virial coefficient was considered in the calculation of K_{SEC}. To elucidate the causes of this artefact, the K_{SEC} vs. K_o dependences calculated from Eqs. (10) and (11) were plotted in Fig. 1 for various values of the volume fraction φ. Fig. 1 shows that for $\varphi = 0.5$ and $\varphi = 1.0$ the calculated dependences pass through a distinct maximum which exceeds in a wide range of K_o the value $K_{SEC} > 1$. Since for flexible chain macromolecules which strongly swell in a thermodynamically good solvent φ reaches considerably high values already at relatively low weight concentrations (depending on molecular weight), it is obvious that for swelling macromolecules this model is nonrealistic. The physically unreal K_{SEC} values may result from the calculation also for a relatively low-molecular weight polymer (cf. PS 111 000), if K_o is high.

4. A shortcoming of the model based on a change in effective dimensions consists in that the $c_2 = c_p$ (concentration in the supernatant equals that in the pore). The Anderson and Brannon model does not introduce this assumption.

5. Thus, the results of measurements of the distribution coefficients under stationary conditions are not very encouraging with respect to the possible

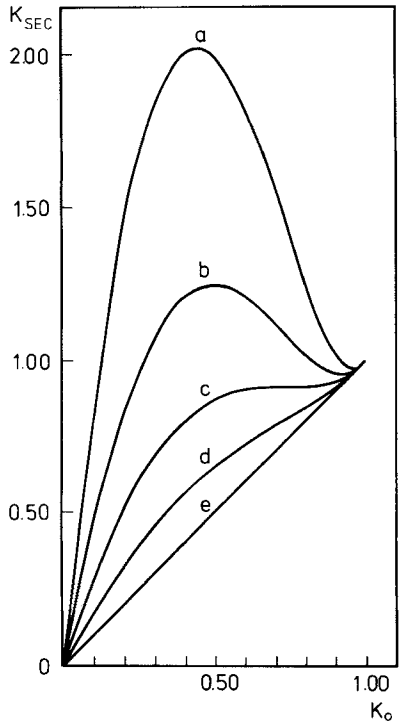

Fig. 1.
The K_{SEC} vs K_o functions for various values of volume fractions φ

a: φ = 1.0, b: φ = 0.5, c: φ = 0.25, d: φ = 0.1, e: φ = 0.

comparison with the dynamic chromatographic experiment. A question remains whether it is possible to rule out or evaluate the contribution of secondary (nonexclusion) interactions. It is quite probable that also kinetic aspects (i.e. the sorption-desorption rate) in the dynamic chromatographic process may become operative. The Anderson-Brannon model was worked out for the behaviour of rigid particles in a porous medium, which is a limiting factor of its suitability

in the case of a coil of the flexible chain macromolecule. On the other hand, however, the calculations of Casassa (8) and Giddings (9) demonstrate that differences between the behaviour in the SEC of statistical coils and rigid spherical macromolecules are not too drastic. Although our attempts to quantify exactly the role played by the mechanisms and models described above in the elucidation of concentration effects in the SEC of macromolecules have failed, our previous conclusions regarding the effect of dynamic viscosity effects remain valid (10).

It is difficult to draw conclusions, as further investigation of the problem of distribution coefficients under stationary conditions. Undoubtedly, further development of the Anderson-Brannon model for the case of macromolecules other than rigid ones would be useful. From the experimental point of view, it is desirable to investigate also other types of macromolecuIs, and rigid particles in the first place. Both theoretical research and experiments in this direction will continue.

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