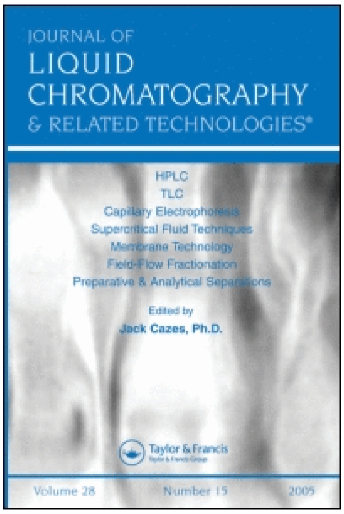

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J. Jančа[a]; S. Pokorný[a]; M. Bleha[a]; O. Chiantore[ab]
[a] Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague 6, Czechoslovakia [b] Visiting from Istituto di Chimica Macromolecolare dell'Universita di Torino, Torino, Italy

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CONCENTRATION EFFECTS IN SIZE EXCLUSION CHROMATOGRAPHY UNDER EQUILIBRIUM STATIONARY CONDITIONS

J.Janča*, S.Pokorný, M.Bleha and O.Chiantore+
Institute of Macromolecular Chemistry, Czechoslovak
Academy of Sciences, 16206 Prague 6, Czechoslovakia

ABSTRACT

In size exclusion chromatography the elution volume increases with increasing concentration of injected polymer solutions. Several particular processes contribute to this concentration effect. Under model equilibrium stationary conditions, where the phases remain immobile, it is possible to eliminate dynamic phenomena connected with the higher viscosity of polymer solution in the chromatographic zone. The only factors operative here are the effect of changes in the effective size of macromolecules in solution with a change in concentration and the effect of the varying accessible pore volume with varying concentration. The ratio of these two contributions has been investigated both theoretically and experimentally. Theoretical calculations indicated that with both phenomena operating simultaneously, the elution volumes may increase or decrease with increasing concentration according to the given experimental conditions, as a result of the coupled influence of molecular parameters of the samples under study, of the thermodynamic quality of the solvent and of the distribution coefficient on the given porous material. Experimental results showed, however, that at least under stationary conditions secondary exclusion is probably operative to a less important degree.

INTRODUCTION

Separation in gel permeation chromatography (GPC) is usually explained by the mechanism of a simple steric exclusion. For this reason, especially in recent years,

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+Visiting from Istituto di Chimica Macromolecolare dell'Università di Torino, Via G.Bidone,36, 10125 Torino, Italy the method has been frequently called size exclusion chromatography (SEC). If no further interactions appear between separated molecules and the porous structure of the stationary phase, such as, e.g., adsorption or incompatibility etc., the retention characteristics of the given column packing (e.g., distribution coefficient or elution volume) are only a function of the effective size of separated molecules or of the ratio of the size of molecules to the pore size of the column packing. In the separation of polymers by the SEC method, even with the predominating mechanism of steric exclusion, some other effects are distinctly operative. They ensue from the properties of solutions of high-molecular weight compounds, are related to the concentration, overall injected amount and flow rate of the mobile phase, and considerably affect retention.

These so-called concentration effects in most cases impair the separation efficiency, and if unjustifiably neglected, may even lead to a wrong quantitative interpretation of experimental chromatographic data. Although the existence of the concentration effects has been observed experimentally virtually starting from the introduction of the SEC method, many contradictory explanations of these phenomena have been reported, while any quantitative treatment of the problem was completely missing. Several earlier papers (1-7) contain a detailed experimental investigation of the concentration effects in SEC; in three of them (1,4,7), a completed theoretical model is offered, making possible a quantitative description of the phenomena. Under conditions where no changes in the pore size with varying thermodynamic quality of the mobile phase in the zone of the eluting sample take place, there may be operative three main contributions to the overall change in the elution volume of a monodisperse polymer due to the concentration effects. These are a contribution caused by a change in the effective size of separated macromolecules with concentration, a contribution of the so-called secondary exclusion, i.e. of the reduction of the accessible pore volume of the column packing by the proper volume of the separated macromolecules which logically increases with increasing concentration, and finally a contribution due to the lower mobility of the viscous polymer solution compared with the pure solvent of the mobile phase, called the viscosity phenomenon.

A preceding theoretical analysis (4,7) has revealed that the latter contribution (viscosity phenomenon) predominates; under concrete experimental conditions, it causes some 80-90% of the total change in the elution volumes depending on concentration. Using dynamic chromatographic experiments, it is difficult to estimate, however, what proportions there are between the contribution caused by a change in the effective size of macromolecules with varying concentration and the contribution of secondary exclusion. Some way for the experimental and theoretical verification of the mutual effect of these contributions is provided by the static experiment. In this arrangement, a known quantity of porous material (packing) is mixed with a certain amount of polymer solution of known concentration. After the state of equilibrium has been reached, it is possible to evaluate the individual contributions using concentration changes of the polymer solution outside the porous material. A change in the concentration of polymer solution occurs each time when macromolecules in solution cannot penetrate at least into one part of the pores, in contrast with molecules of the solvent. Some authors have already described this type of the experiment. Haller (8) studied the elution of benzyl alcohol and some viruses on porous glass in a dynamic chromatographic system and then the kinetics of penetration in a stationary arrangement, but he did not observe any change in concentration with large molecules excluded in SEC. For partly penetrating molecules he found that rather a long time was needed for the establishment of equilibrium (some 50 min.). Similar results were obtained by De Ligny (9). Chang (10) found that the volume of molecules excluded on the given gel in the static experiment increases linearly with the logarithm of molecular size, independently of concentration. Yau et al. (11) and Yau (12) carried out measurements under stationary conditions, using silicagel and polymeric gel (Styragel). They came to a conclusion that the exclusion mechanism is probably stronger on porous silicagel. Grubisic and Benoit (13) determined the overall pore volume and distribution by employing the static and dynamic methods and porosimetry. According to their results, the concentration had no demonstrable effect on the distribution coefficient determined under static conditions. However, the basic questions concerning the effect of concentration on the results of the static experiment have not yet been studied.

THEORY

The elution volume of a monodisperse polymer, V_e, in SEC may be expressed as

$$V_e = V_i + K_{SEC} . V_p \qquad (1)$$

where V_i is the interstitial volume, V_p is the pore volume and K_{SEC} is the formal distribution coefficient. If the volume V_1 of a solution of the partly permeating polymer having the concentration c_1 is mixed with a porous material, the solvent penetrates into all pores, while the polymer penetrates only into accessible pores, thus concentrating in the volume V_2 of solution outside the pores and in the accessible pores. Hence, the original concentration is increased and becomes the eventual concentration c_2. For the mass balance the relationship

$$c_1V_1 = c_2V_2 \qquad (2)$$

is valid. If the solution volume outside the pores after mixing with the porous material is V_o, we have

$$V_1 = V_o + V_p \qquad (3)$$

Assuming an equal K_{SEC} for the dynamic and static experiment, it also holds that

$$V_2 = V_o + K_{SEC} \cdot V_p \qquad (4)$$

The neglect of volume changes in solution with varying concentration is justified in the range of low concentrations of the solutions under investigation, and also because concentration changes from c_1 to c_2 are not too large. For the ratio of solution concentrations before and after mixing with the porous material we have, then,

$$c_1/c_2 \doteq 1 + V_p/V_1(K_{SEC} - 1) \qquad (5)$$

The dependence of the distribution coefficient K_{SEC} on the effective size of the macromolecule may be expressed through an empirical calibration function (7)

$$K_{SEC} = f(v . \varepsilon) \qquad (6)$$

where v is the volume of the unswollen macromolecular coil (e.g., in the theta solvent), and ε is a dimensionless swelling factor. This function may be expressed, e.g., by a polynomial. The central part of the calibration plot may be successfully approximated also by a straight line (1)

$$K_{SEC} = P + Q \ln(v.\varepsilon) \qquad (7)$$

with the constants P and Q. The swelling factor ε depends on the concentration c according to Rudin and Wagner (14)

$$1/\varepsilon = 1/\varepsilon_o + c/c_x(\varepsilon_o - 1)/\varepsilon_o \qquad (8)$$

where ε_o is the swelling factor at infinite dilution (c=0) and c_x is the critical concentration at which $\varepsilon = 1$. These quantities may be calculated using the following relations

$$\varepsilon_o = [\eta] / [\eta]_\theta = K.M^{a-0.5}\, M_o^{3/2}/0.25\, \beta^3 \qquad (9)$$

where $[\eta]$ is the intrinsic viscosity defined by the Mark-Houwink equation

$$[\eta] = K.M^a \qquad (10)$$

with the constant K and the exponent a, M_o is half the molecular weight of the monomer repeating unit of the vinyl polymer, β (in Å) is the effective bond length. $[\eta]_\theta$ is the intrinsic viscosity in the theta solvent, which may be determined from

$$[\eta]_\theta = K_\theta.M^{0.5} \qquad (11)$$

where

$$K_\theta = 2.5\text{x}10^{-1}\, \beta^3/M_o^{3/2} \qquad (12)$$

It also holds that

$$c_x = \varphi_x M/N_o v \qquad (13)$$

and

$$v = 4\pi [\eta]_\theta M/3\Phi' \qquad (14)$$

where N_o is the Avogadro constant, φ_x is the critical volume fraction corresponding to the concentration c_x at which the size of macromolecules in solution is the same as under the theta conditions, the Flory constant is $\Phi' = 3.1\mathrm{x}10^{24}$. If the concentration affected only the change in the effective size of macromolecules, the resulting c_1/c_2 would depend on concentration according to the relation

$$c_1/c_2=1+V_p/V_1\{P+Q\ \ln v-Q\ \ln[1/\varepsilon_o+c_2(\varepsilon_o-1)/c_x\varepsilon_o]-1\} \qquad (15)$$

obtained by substitution from Eqs (7) and (8) into Eq. (5). If one bears in mind also the effect of secondary exclusion, it is necessary to subtract the volume occupied by macromolecules in solution from the available pore volume determining the distribution coefficient K_{SEC}.

$$K'_{SEC} = K_{SEC}(1 - \varphi) \qquad (16)$$

For the given concentration, the volume fraction in Eq. (16) can be calculated using the equation

$$\varphi = c.N_o.v.\ \varepsilon/M \qquad (17)$$

EXPERIMENTAL

Polystyrene (PS) standards (Waters Associates, Inc., Milford, Mass., USA) with a narrow molecular weight distribution were used in the investigation. The molecular parameters of these standards are summarized in Table 1. A known quantity of solutions of the PS standards of a known concentration in tetrahydrofuran (THF) purified by distillation was mixed with a known quantity of silicagel Porasil DX(Waters), particle diameter 75-125 μ, extracted with THF and methanol and dried in vacuo. The mixture was shaken in a septum-closed flask for 2-24 h, in order to achieve an equilibrium distribution of the solvent and solute between the phases. After that, the mixture was left for a short time (a few minutes) at rest, for silicagel to be able to sediment, and the solution over silicagel was taken for the determination of the change in the concentration of the dissolved PS standard. The change in concentration was assessed by comparing the areas of chromatograms of the original solution and of the solution after interaction (mixing) with silicagel, which were injected on a column with low exclusion limit.

The calibration curve of Porasil DX needed for further calculations was determined by measurements carried out on a series of the PS standards under dynamic chromatographic conditions. For this purpose, a column 250x4.6 I.D. in size was packed with Porasil DX. The measurement was performed using an HP 1084 B (Hewlett-Packard, Palo Alto, Ca., USA) liquid chromatograph; an HP 1030B UV detector, wavelength 254 nm, was used in the detection. The flow-rate of the solvent (THF) was 0.2 ml/min, the injected amounts of the PS standards were 10 μl, at concentrations of approx. 0.01% (w/v). At such low concentrations the concentration effects under the given conditions are already operative in an extent comparable with experimental error.

The total pore volume of a known weight amount of Porasil DX was determined by means of the static experiment using the totally excluded PS standard and benzene and employing Eq.(5), and also by mercury porosimetry. The porous characteristic was measured with a Porosimeter Mod.225 (Carlo Erba Strumetazione, Italy) apparatus. The penetration of mercury into the sample was investigated at pressures 0-2x10^5 kPa, in agreement with the extent of the pore diameters up to 8 nm. The measured values were treated mathematically by means of an IBM 1370-1395 computer. In this way the total pore volume was determined.

Before measurements by the method of mercury porosimetry, the silicagel sample was dried at the pressure 0.6 kPa and room temperature for 15 h. After being transferred into the measuring dilatometer, it was degassed at 6 Pa for 1 h, and after that covered with mercury at the same pressure.

RESULTS AND DISCUSSION

Using Eqs (15),(16) and the known molecular parameters summarized in Table 1, we calculated the theoretical dependence of the concentration ratio of the original solution of the PS standard and of the solution obtained after mixing with silicagel, c_1/c_2, on the resulting equilibrium concentration c_2. The standards PS 3 and PS 6, both situated in the linear part of the experimentally determined calibration curve in Fig.1, were chosen for the calculations. The linear regression method was employed in calculating the constants of Eq.(7), P = -4.6746 and Q = -0.1303, for this part of the calibration curve; the regression coef-

TABLE 1

Molecular Parameters of Polystyrene Standards and Calibration Dependence of Silicagel Porasil DX

Polystyrene standard	Molecular weight$^{(a)}$	$K_{SEC}^{(b)}$	$v \times 10^{18}$	$1/\varepsilon_o$	$(\varepsilon_o-1)/(c_x\varepsilon_o)$ at $c_x=1$	$\ln(v.\varepsilon_o)$
PS 1	2 700 000	0.000	486.6	0.2790	78.26	-33.98
PS 2	867 000	0.023	88.55	0.3570	39.55	-35.93
PS 3	694 000	0.094	63.42	0.3747	34.41	-36.32
PS 4	470 000	0.096	35.34	0.4077	26.82	-36.98
PS 5	200 000	0.358	9.811	0.4908	15.04	-38.45
PS 6	110 000	0.492	4.002	0.5588	9.668	-39.48
PS 7	33 000	0.729	0.6575	0.7256	3.293	-41.54
PS 8	10 000	0.899	0.1097	0.9402	0.3951	-43.59
Benzene	-	1.000	-	-	-	-

(a) A manufacturer s data; (b) measured under dynamic conditions.

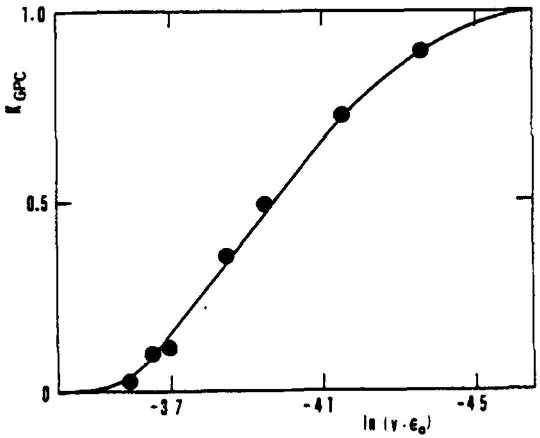

FIGURE 1. Calibration plot of silicagel Porasil DX

ficient was R = 0.995. The concentrations ranged from 0 to 2.5% (w/v). The Mark-Houwink equation (15),

$$[\eta] = 1.17\text{x}10^{-2}\ M^{0.717} \tag{18}$$

valid for PS in THF at 25°C, and $\hat{\varepsilon}$ = 4.96 Å (14) were used in the calculations. The chosen V_p/V_1 ratio was 1/4. The results of the calculations are shown in Fig.2 as the dependence of the change in c_1/c_2 expressed in per cent with respect to zero concentration on the concentration c_2.

$$\Delta\,(c_1/c_2) = \frac{(c_1/c_2)_{c_2} - (c_1/c_2)_{c_2=0}}{(c_1/c_2)_{c_2=0}} \times 100 \tag{19}$$

The hypothetic value $(c_1/c_2)_{c_2=0}$ at c_2=0 was calculated from Eq.(5). Solid curves in Fig.2 correspond to a case where only the effect of a change in the effective size of macromolecules with varying concentration

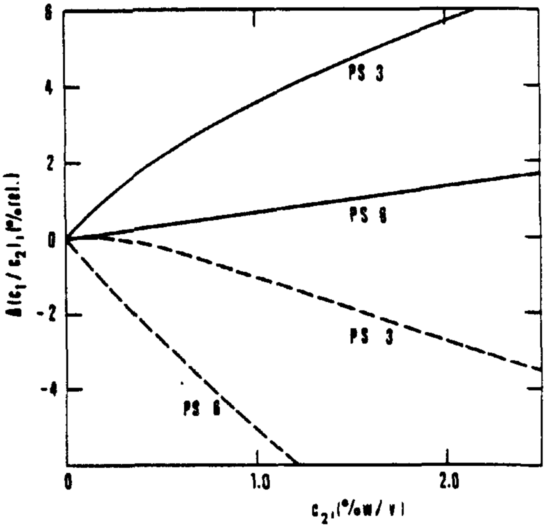

FIGURE 2. Theoretical dependence of the relative change in the concentration ratio of the original solution of polystyrene standards and of the solution on mixing with silicagel on equilibrium concentration.
——— change in the effective size of macromolecules only;
------ change in the effective size and secondary exclusion.

is operative. As supposed both solid curves have an increasing tendency; the curve of the PS standard with the higher molecular weight has a steeper slope. The shape of the curves is easy to understand, because with increasing concentration the hydrodynamic volume of macromolecules decreases, K_{SEC} increases, and consequently the ratio c_1/c_2 also increases. If also the effect of secondary exclusion is considered, the dependence $\Delta(c_1/c_2)$ on c_2 represented in Fig.2 with broken curves is more complicated. For PS3, the curve in Fig.2 first slightly increases from zero concentration to approximately $c_2 = 0.2\%$; in other words, in this range the effect of the change in the effective size of macromolecules with varying concentration slightly predominates over the effect of secondary exclusion. In the range of higher concentrations above $c_2 > 0.2\%$ the effect of secondary exclusion begins to prevail, and the function in Fig.2 decreases. For the standard PS6 within the whole concentration range under investigation the effect of secondary exclusion strongly predominates, and the curve in Fig.2 steeply decreases. It can be seen from the theoretical analysis presented above, that under the given experimental conditions both contributions to the concentration effects considered in this case may be operative simultaneously in a rather complex way. The dependence of $\Delta\ (c_1/c_2)$ on c_2, and hence also the participation of each of the contributions considered here to the concentration effect will therefore have to be examined for each particular case (i.e. molecular weight, thermodynamic quality of the solvent, distribution coefficient etc.) using the respective quantitative relations given in the Theoretical Part, and especially by means of Eqs (15) and (16). It should be stressed, however, that the dependence in Fig.2 holds only assuming the validity of Rudin´s theory (14), i.e. in the range of dilute solutions. Another condition for the validity of the dependence in Fig.2 is that under stationary conditions the assumed mechanism of secondary exclusion becomes quantitatively operative to an extent corresponding to the derived relations.

The summarized experimentally determined c_1/c_2 values for various original concentrations c_1 (and consequently various equilibrium concentrations c_2 after interaction with silicagel) within the concentration range from approximately 0.03% to 4% (w/v) are given in

Table 2. Using Eqs (15) and (16), we calculated the theoretical c_1/c_2 values for all experiments, particularly for a case where only the change in the hydrodynamic volume of macromolecules with concentration is operative, and for a case where secondary exclusion is also operative simultaneously and to the full extent. The calculations were carried out using experimental c_2 values. The total pore volume was calculated from the known weighed amounts of silicagel and from the specific porosity values, i.e. from the pore volume per one gram of silicagel, determined from c_1/c_2 for a totally excluded standard PS 1 (V_{spec} = 0.736 ml/g), and from mercury porosimetric measurements (V_{spec} = 0.826 ml/g). The value V_p = 0.826 ml/g was eventually used in the calculations, as it could not be ruled out that the low--molecular weight fractions of the excluded PS standard partly penetrated into pores in dynamical chromatographic determination. The ratio of the $(c_1/c_2)_{exp}$ values determined experimentally for various concentrations to the $(c_1/c_2)_{cal}$ values calculated as described above should be unity in those cases where the theoretical calculation agrees with the experiment. Results in Table 2 indicate that in both cases, i.e. if only the effect of changes in the size of macromolecules with concentration or also secondary exclusion are considered for both PS standards, the ratio $(c_1/c_2)_{exp}:(c_1/c_2)_{cal}$ increases with increasing concentration. Since the steeper rise, and thus larger deviations from unity are evident for the latter case, where the simultaneous effect of secondary exclusion is taken into account, it seems that secondary exclusion is not operative, at least under secondary conditions. The increasing trend of $(c_1/c_2)_{exp}:(c_1/c_2)_{cal}$ with increasing concentration is however reflected also in the case where only the in-

TABLE 2

The Effect of Concentration on the Ratio of the Concentration of the Original Solution of Polystyrene Standard to the Equilibrium Concentration on Mixing with Silicagel

Concentration c_1 (%)	Weight of silicagel (g)	c_1/c_2 exp.	V_1 (ml)	$(c_1/c_2)_{exp}:(c_1/c_2)_{calc.}$ $A^{(a)}$	$B^{(b)}$	K_{SEC} $C^{(c)}$	$D^{(d)}$
			Polystyrene standard PS 3				
4.21	1.3868	0.9109	4	1.15	1.32	0.689	0.273
3.11	1.7026	0.8802	5	1.12	1.25	0.574	0.246
1.41	1.2915	0.8390	4	1.07	1.14	0.396	0.179
1.13	1.1676	0.8377	4	1.05	1.10	0.327	0.162
0.52	1.5692	0.7359	4	1.03	1.07	0.185	0.122
0.125	0.7868	0.7023	2	1.00	1.01	0.084	0.077
			Polystyrene standard PS 6				
2	0.5215	0.9642	2	1.08	1.16	0.834	0.509
1	0.5821	0.9330	2	1.06	1.12	0.721	0.492
0.5	0.6284	0.8885	2	1.03	1.06	0.570	0.482
0.125	0.6737	0.8765	2	1.03	1.04	0.556	0.473
0.03125	0.5465	0.8834	2	1.00	1.01	0.483	0.470

[a] Change in the size of macromolecules only; [b] change in the size of macromolecules and secondary exclusion; [c] measured under stationary conditions; [d] calculated from Eqs (15) and (7).

fluence of a change in the hydrodynamic volume with concentration is considered. This finding means that on mixing the polymer solution with silicagel the real concentration c_2 at higher concentrations is lower than corresponds to the theoretical model.

In other words, the experimentally determined distribution coefficient K_{SEC} calculated from Eq.(5) increases with concentration much more quickly than the coefficient calculated entirely theoretically from Eqs (15) and (7) (cf.Table 2).

Several conclusions may be drawn from our findings. In the range of slightly concentrated and concentrated solutions, the hydrodynamic volume does not delimit the volume inaccessible to parts of chains of other macromolecules. For this reason, at least in slightly concentrated and concentrated solutions, Eq.(17) does not adequately describe the volume fraction which must be subtracted from the accessible pore volume. In the case of mutual penetration of coils, however, the individual macromolecules still behave statistically as discrete coils and not as aggregates, because K_{SEC} does not decrease, but on the contrary increases continueously with increasing concentration. The resulting K_{SEC} value is to a certain extent determined by the gel pore size distribution, but also by the ratio of the total volume of the accessible pore to the volume in which the coil cannot approach the pore wall at a distance smaller than the coil radius (16). Finally, the possibility cannot also be ruled out that macromolecular coils are infact more coiled than is assumed by the theoretical model of the dependence of the hydrodynamic model on concentration. However, with respect to the good quantitative agreement between the theory (14) and experiment in the range of dilute solutions, the latter factor is a minority one.

An answer to the question to what extent secondary exclusion is operative may be provided by a stationary experiment in the theta-solvent, or a dynamic chromatographic experiment in the theta-solvent. An investigation of further quantitative aspects of these conclusions is in progress.

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