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CONCENTRATION EFFECTS IN SIZE-EXCLUSION CHROMATOGRAPHY OF POLYMERS

SEPARATION OF THE CONTRIBUTIONS FROM VISCOSITY AND HYDRODYNAMIC VOLUME CONTRACTION

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SUMMARY

A practical experimental procedure is suggested for the separation and quantification of the contributions from viscosity and from hydrodynamic volume contraction to the concentration dependence of retention volumes in size-exclusion chromatography of polymers. Application of the method to the elution of polystyrene in a good solvent showed that the viscosity is generally not the main cause of the total concentration effect. The opposite is true for poly(methyl methacrylate) in an ideal solvent; however, in this case, some other effect beside viscosity is also operative.

INTRODUCTION

The concentration dependence of polymer distribution coefficients in size-exclusion chromatography (SEC) is a well-known experimental phenomenon whose origin has been attributed either to the sole effect of the contraction of macromolecular coils with increasing polymer concentration[1] or to the concurrence of the latter process with others, such as the frictional forces acting on the solute molecules during the flow and, possibly, the "secondary" exclusion of the macromolecules from the pores of the packing when the pore volume is already "filled" with solute[2].

The effect of polymer concentration increases with increasing molecular weight and solvent "goodness". The existing semi-empirical and theoretical models proposed for the interpretation of concentration effects in SEC mainly correlate the change of coil size[1] and the thermodynamic quality of the solvents[3] with the increase of polymer concentration. Only one theory of concentration effects explicitly also takes into account the contributions from viscosity and secondary exclusion[2]. From the application of this theory to experimental results, it has been estimated that the major contribution to the total concentration dependence of polymer distribution coefficients is from the viscosity of the solutions.

The practical evaluation of the different contributing phenomena to the concentration effect in SEC is a difficult task, because in most cases all the possible processes will be operative at the same time. However, under particular and well-

defined experimental conditions, one should be able to isolate and therefore determine some single effect. The viscosity contribution, for instance, is operative in the column interstitial volume only, and consequently the elution of samples excluded from the pore volume should be affected only by the viscosity effect. Another case where only the viscosity of the injected solutions would be responsible for the changes of the distribution coefficients is that of polymer solutes eluted in thermodynamically ideal solvents, where the molecules are in their unswollen, unperturbed dimensions. On the contrary, in real chromatographic experiments, when polymer molecules flow through the columns, coil contraction alone has never been shown to take place without any contribution from the frictional forces. To reach such a limiting condition with polymer solutions in the presence of a porous packing, distribution coefficients under static conditions should be measured.

The contribution due to secondary exclusion has been investigated under stationary conditions, and the results showed that this process is not likely to have much influence on the overall concentration effect[4]. Therefore, it might be concluded that the effects of macromolecular coil contraction and of solution viscosity are mainly responsible for the total increase of polymer distribution coefficients in SEC. In this paper, we report a practical experimental procedure[5] for the separation of these two effects in the case of elution of polymer molecules permeating the chromatographic pore volume. The method is suggested for a tentative evaluation of the relative contribution of the two effects over the total concentration dependence, and it could be seen also as a check on the presence of other processes, not explicitly taken into account[6]. The application of the method to some real systems is discussed.

METHOD

The SEC distribution coefficient is given by

$$K_{SEC} = (V_R - V_0)/V_P \tag{1}$$

where V_R is the solute retention volume, V_0 the interstitial volume and V_P the pore volume. Eqn. 1 is formally defined for the case where no secondary effects are operative.

At non-zero polymer concentration, when concentration effects appear, it is true that

$$K'_{SEC} = K_{SEC} + \Delta K \tag{2}$$

Under our basic assumption that only size contraction and viscosity are effectively contributing to the concentration effect, we may write

$$\Delta K = f(V_h, \eta_{sp}) \tag{3}$$

where V_h is the hydrodynamic polymer volume and η_{sp} is the polymer specific viscosity.

Both parameters of eqn. 3 are concentration dependent; the specific viscosity is calculated by the Huggins equation

$$\eta_{sp} = [\eta] \, c + k_H [\eta]^2 \, c^2 \tag{4}$$

where c is the concentration and $[\eta]$ is the limiting viscosity number of the polymer; and the hydrodynamic volume through the following relations

$$V_h = \text{const.} \langle R^2 \rangle_0^{3/2} \, \alpha^3 \tag{5}$$

$$\alpha^3 = f(c) \tag{6}$$

$\langle R^2 \rangle_0$ is the square of the unperturbed end-to-end distance of the macromolecule, and α is the linear expansion factor, which decreases with increasing concentration[7].

In general, if polymer concentrations are not too high, a linear dependence of the measured distribution coefficients on concentration is found.

From the injection of totally excluded polymer molecules at different concentrations, the increment of distribution coefficients due to the viscosity of the solutions, $\Delta K(\eta_{sp})$, can be measured, as long as the reduction of the coil size is not sufficient to allow penetration of the macromolecules into the gel pores. It has been shown[8] that for excluded polymers a linear relationship exists between the specific viscosity of the injected solution and the distribution coefficient, up to viscosity values where the solution behaviour is still Newtonian. Now the assumption is made that for polymer solutions with the same specific viscosity, the same viscosity contribution results, independent of the fact that the polymer molecules can diffuse into the gel pores. Therefore, for each injected sample eluted in the column permeation range, the total increase of the distribution coefficient at each given concentration may be expressed as

$$\Delta K = \Delta K(\text{size}) + \Delta K(\eta_{sp}) \tag{7}$$

$\Delta K(\eta_{sp})$ may be evaluated from the value of the excluded polymer at the same specific viscosity. The specific viscosities of the polymers are calculated through eqn. 4, and the amount of the viscosity effect may be subtracted from the measured ΔK to obtain the contribution due to the coil contraction.

The polymer concentrations used in the application of the method are the nominal injected concentrations. This means that the sample dilution occurring in the columns is neglected. This approximation is reasonable if the dilution affects, almost equally, the hydrodynamic volume and viscosity dependence of the distribution coefficients.

MATERIALS

Polymer distribution coefficients as a function of injected concentrations have been measured with narrow-distribution polymer standards and different high-performance silica gel column systems, packed in our laboratory. Each column was 25 cm × 0.46 cm I.D. Column system I consisted of two columns, packed with 100 nm and 50 nm nominal pore size, respectively. In system II two columns, both having 10 nm mean pore size, were used. System III was similar to system II, but one of the columns was prepared with a different silica gel, having approximately the same pore size of 10 nm but a larger pore volume.

Polystyrene (PS) standards were obtained from Arro Labs. (Joliet, IL, U.S.A.) and from Waters Assoc. (Milford, MA, U.S.A.); poly(methylmethacrylate) (PMMA) standards were from Polymer Labs. (Shrewsbury, U.K.). PS samples were eluted in tetrahydrofuran (THF), whereas for PMMA samples 2-ethoxyethanol was employed. Injection volumes were 10 μl. A UV photometer at 260 nm was employed for detection of PS samples, and a differential refractometer for the PMMA sample. The flow-rate was 0.5 cm^3/min.

RESULTS AND DISCUSSION

The retention behaviour of PS in THF has been investigated as an example of polymer molecules dissolved in a good solvent, where both the viscosity and the coil contraction are acting at the same time. Column systems I and II were employed, covering different molecular-weight ranges. In both systems, polymer samples completely excluded from the SEC columns were eluted, together with other samples partially permeating the gel pores. The molecular weights of the excluded samples were, for the two column systems, 10^7 and 4.7 · 10^5, respectively. At increasing polymer concentrations, the chromatographic distribution coefficients K'_{SEC} increased, and distorted chromatograms were also obtained for the high-molecular-weight samples. When the asymmetry and distortion of the chromatograms was severe, retention volumes V_R of the polymer samples were obtained by measuring the first statistical moment of the peaks.

K_{SEC} values for the different polymer samples were obtained by extrapolation of the measured distribution coefficients K'_{SEC} to zero concentration. The resulting ΔK values are plotted against the polymer concentrations in Figs. 1 and 2 for column
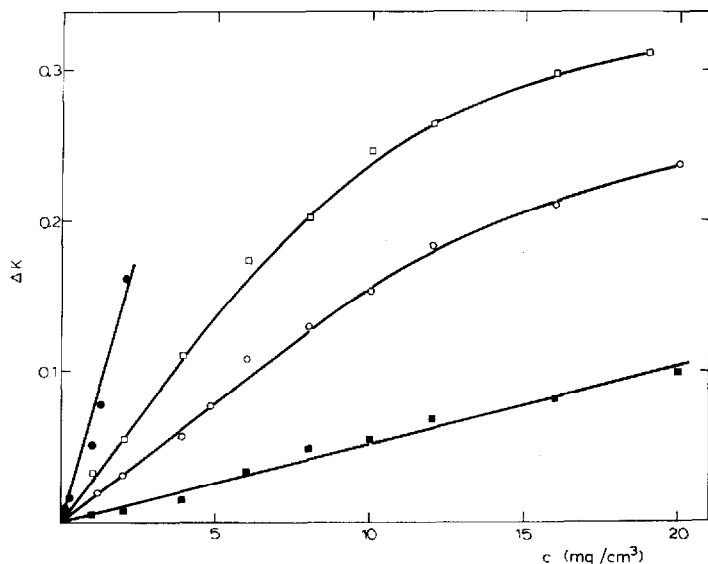

Fig. 1. Increment of distribution coefficients with concentration for PS samples in THF on column system I. Molecular weights: ●, 10^7; □, 670 000; ○, 200 000; ■, 17 500.
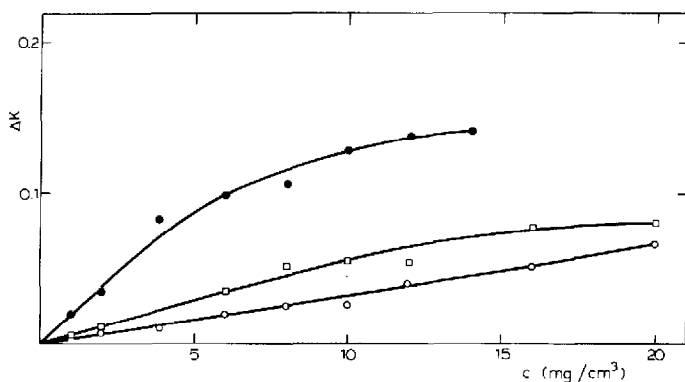
Fig. 2. Increment of distribution coefficients with concentration for PS samples in THF on column system II. Molecular weights: ●, 470 000; □, 50 000; ○, 17 500.

system I and II, respectively. The concentration dependence of ΔK increases with polymer molecular weights, as expected, and is linear either at low concentrations or in the whole conentration range, depending on the molecular weights.

From eqn. 4, the specific viscosities of the PS samples could be evaluated. Huggins constant (K_H) values for PS in THF were interpolated from data in the literature[9], and limiting viscosity numbers $[\eta]$ were obtained from the equation[10]

$$[\eta] = 1.11 \cdot 10^{-2} M^{0.723} \tag{8}$$

Therefore, the total increment of distribution coefficients, ΔK, can be plotted against the polymer specific viscosities, as shown in Figs. 3 and 4.

The ΔK values for the two excluded PS samples are due to the viscosity effect taking place in the interstitial volume. For the permeating polymer molecules at each specific viscosity, ΔK values higher than those of the excluded polymer are found, suggesting that, in addition to viscosity, some other effect is also operative. The increase in ΔK is higher for the lower-molecular-weight samples, as these polymers have, for the same η_{sp} value, a higher concentration.

For each of the permeated polymer molecules, the contribution from viscosity, $\Delta K(\eta_{sp})$, to the total concentration effect is given by the ΔK of the excluded polymer at the same η_{sp} value. The $\Delta K(\eta_{sp})$ values, obtained for polymer samples eluted in different parts of the column separation range for the two investigated column systems, are plotted in Fig. 5 against the injected solution concentrations: an initial linear dependence is clearly evident. The ratio of the slopes in Fig. 5 over the ones of the corresponding samples in Fig. 1 or Fig. 2 give an estimate of the fractional amount of the contribution from viscosity over the total concentration dependence of distribution coefficients measured in our experimental system. These ratios are reported in Table I, where it is evident that the effect of viscosity appears to be responsible for 20–30% only of the total distribution coefficient change in column system I, and for 50–60% in column system II.

Under our assumption that only viscosity effects and hydrodynamic volume contraction occur, the above results show that for polymer molecules dissolved in a
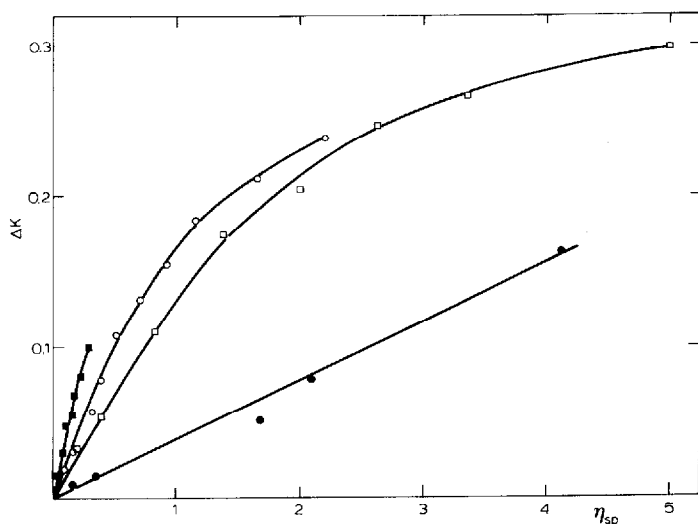
Fig. 3. Increment of distribution coefficients with polymer specific viscosities for PS in THF on column system I. Symbols as in Fig. 1.

TABLE I

RELATIVE CONTRIBUTIONS OF VISCOSITY TO THE TOTAL CONCENTRATION EFFECT FOR PS IN THF

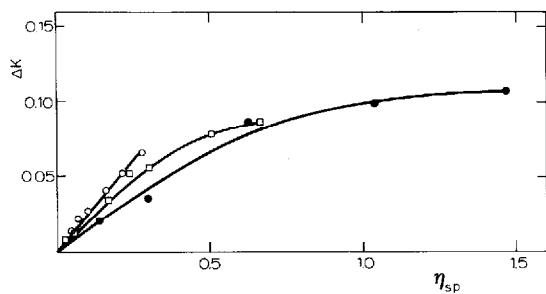| Polymer molecular weight	$d[\Delta K(\eta_{sp})]/d[\Delta K]$	Column system
17 500	0.18	I
	0.53	II
50 000	0.64	II
200 000	0.23	I
670 000	0.29	I

Fig. 4. Increment of distribution coefficients with polymer specific viscosities for PS in THF on column system II. Symbols as in Fig. 2.
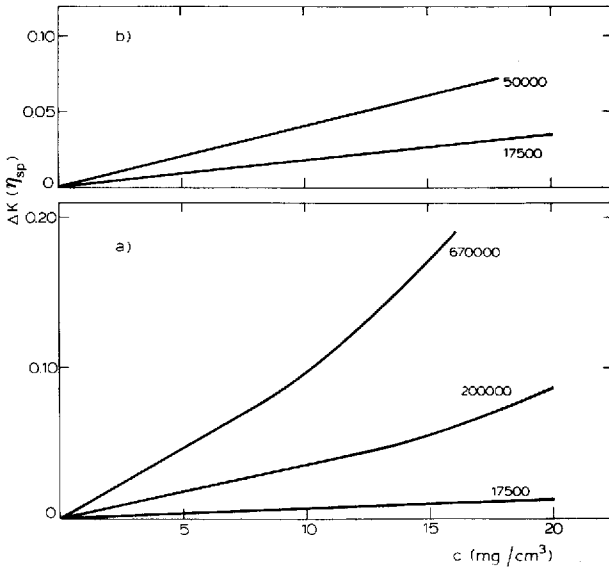
Fig. 5. Viscosity contributions to the change of distribution coefficients with concentration for PS in THF. (a) Column system I and (b) column system II.

good solvent both phenomena are important in determining the total concentration effect, and the relative contributions seem to depend on the column systems.

The concentration dependence of polymer distribution coefficients has also been investigated with solutions of PMMA in 2-ethoxyethanol, which is a practically ideal solvent for the polymer at room temperature[11]. Because of its high polarity, 2-ethoxyethanol is also a convenient eluent for avoiding polymer adsorption on silica gel columns[12].

Distribution coefficients K'_{SEC} have been measured at increasing concentrations
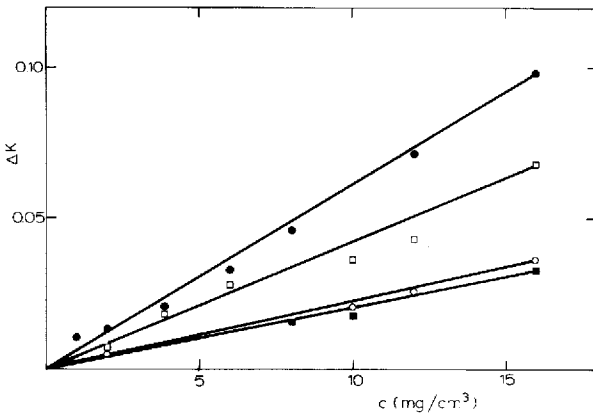

Fig. 6. Increment of distribution coefficients with concentration for PMMA in 2-ethoxyethanol. Samples: ●, 640 000; □, 128 000; ○, 72 000; ■, 45 200.
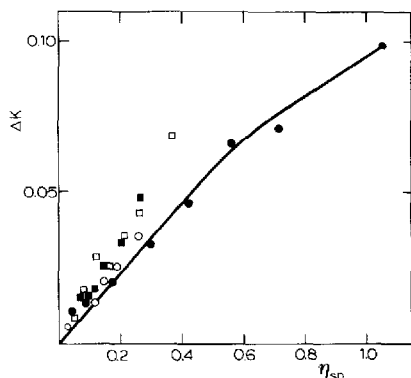
Fig. 7. Increment of distribution coefficients with specific viscosities of PMMA in 2-ethoxyethanol. Symbols as in Fig. 6.

for several narrow-distribution PMMA standards in column system III. PMMA 670000 is the excluded polymer sample. ΔK values relative to these samples are shown in Fig. 6, plotted against polymer concentrations. The increment of distribution coefficients with concentration is lower than that observed in good solvents, and linear dependence between the two variables is again found.

Specific viscosity values have been obtained for the PMMA samples investigated, through the use of the viscometric equation[11]

$$[\eta] = 5.00 \cdot 10^{-2} M^{0.5} \tag{9}$$

and from the value of $k_H = 1$ for the Huggins constant, obtained as an average value by analogy with other values reported for single and mixed ideal solvents of PMMA[13].

The ΔK values *vs.* the polymer specific viscosities are plotted in Fig. 7. The increment of distribution coefficients for the excluded sample, PMMA 640000, is certainly due to the viscosity contribution occurring in the interstitial volume of the columns, and if only such an effect is operative, one would expect that, at the same specific viscosity, all the data relative to the permeating polymers would fall onto the
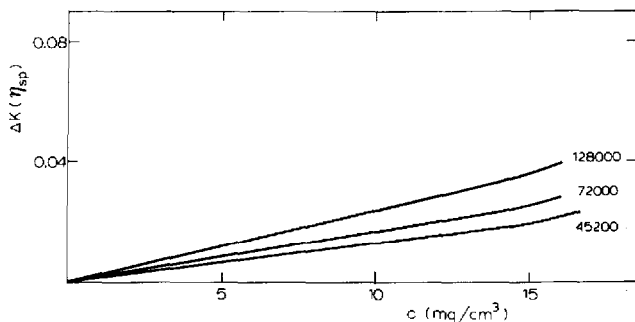

Fig. 8. Viscosity contributions to the change of distribution coefficients with concentration for PMMA in 2-ethoxyethanol.

curve defined by the excluded sample. Fig. 7 shows that for the permeating PMMA samples the increase of distribution coefficients is slightly higher than that relative to the excluded sample. This means that some other effect contributes to the increase of distribution coefficients with polymer concentration.

The viscosity contribution values, $\Delta K(\eta_{sp})$, for different PMMA samples, calculated as previously described, are shown in Fig. 8. From the ratio of the slopes of the straight lines of Fig. 8 and Fig. 6, the average relative contributions to the total concentration effect due to the viscosity are calculated to be in the range 60–80%. Therefore, for PMMA in 2-ethoxyethanol, the main cause of the concentration dependence of the distribution coefficients appears to be the viscosity of the polymer solutions.

There is no straightforward interpretation for the small additional contribution observed in this case, as long as adsorption of the PMMA molecules on the silica substrate can be ruled out when 2-ethoxyethanol is used as eluent[12]. The hypothesis can be made that some other effect, not explicitly taken into account in the present method of treatment of experimental data, is operative. However, it can be suggested that with solutions of polymer molecules in a thermodynamically ideal solvent the effect of increasing the polymer concentration could be comparable to that occurring when the temperature of ideal solutions is decreased and a continuous contraction of the polymer chains below their unperturbed dimensions is found[14]. When such a phenomenon occurs, the flexible macromolecules are seen to shrink below their unperturbed conformation to reach a slightly more compact situation. More data on the macromolecular conformations in semi-concentrated solutions in ideal solvents are necessary to clarify this point.

CONCLUSIONS

Under the assumption that only viscosity and hydrodynamic volume contraction affect the change of the polymer distribution coefficients in SEC, the proposed procedure for treating the experimental data allows the separation and quantification of the two contributions. In some cases, only one of the two contributions is expected; if, by application of the method, the results do not confirm the expectation, evidence is obtained that some other phenomenon is operative.

In the case of PS eluted from silica gel columns by a good solvent, the viscosity is generally not the main cause of the total concentration effect. On the other hand, the opposite happens for PMMA in ideal solvent. However, in this case, an indication that some other effect, besides the viscosity effect, contributes to the overall polymer distribution coefficient change is clearly evident.

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