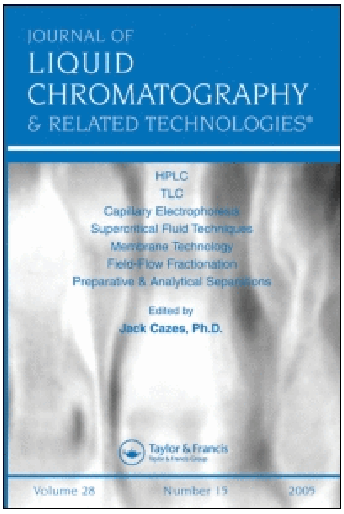

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THE EFFECT OF THE CONCENTRATION AND INJECTED VOLUME OF POLYMER SOLUTION IN SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

The concentration and total injected volume of polymer solution affect elution volumes in size exclusion chromatography. When selecting optimal experimental conditions for both analytical and preparative separation, it must be considered wheter it is more advantageous to inject a smaller volume of solution of higher concentration, or vice versa. Theoretical analysis by means of derived equations and experimental results showed that if the injection conditions are chosen so that the contribution to the total width of the elution curve due to injection width is negligible within the limits of experimental error, and the total amount of the injected polymer is constant, the ratio of concentration to the injected solution volume may be chosen within broad limits without affecting the results of separation to any considerable extent.

INTRODUCTION

Important experimental factors which should be considered in choosing optimal experimental conditions in size exclusion chromatography (SEC), particularly in the separation of polymers, are the concentration

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and total injected volume of polymer solution. In analytical separations it is important to reach a high-quality signal of the detector used, i.e. a sufficient difference between the detector response to the eluating polymer solution, on the one hand, and the noise of the base-line on the other, to make results of the calculation of molecular weight distribution sufficiently reproducible. In preparative separation it is desirable to have the highest possible amount of the fractionated polymer sample in one injection, so that it would not be necessary to inject a limited amount of the polymer repeatedly, which makes the fractionation considerably more labour- and time-consuming. It is generally known that the injection of the sample must be as short as possible, or in other words, the injected volume of polymer solution must be as small as possible to prevent an additional broadening of the elution curve. In such a case the concentration must be sufficiently high to satisfy the requirement on the detector response. It is also well known that with increasing concentration of the injected polymer solution the elution volume also increases. This phenomenon is usually called the concentration effect and has been investigated by many authors (cf.ref.1). A complete theoretical model quantitatively describing concentration effects in the SEC of polymers has been reported in an earlier paper (1). Hence, for experimental purposes one must choose an optimal compromise between the contradictory requirements just outlined.

Let us start with the maximal acceptable injected volume of polymer solution. For the overall width of the elution curve, w_T, we have, according to the rule of additivity of second central moments or variations

$$w_T^2 = w_I^2 + w_P^2 + w_S^2 \tag{1}$$

where w_I is a contribution due to injection, proportional to the injected volume, w_P is a contribution due to the polydispersity of the sample, and w_S is a contribution due to spreading in separation columns, connecting capillaries and in the detector.

Instead of w, the width of the chromatogram may alternatively be described by using the standard deviation, σ. If the elution curve and injection function may be approximated by the Gaussian curve, the width of each contribution, w, is equal to fourfold standard deviation σ. If we consider a certain per cent experimental error in SEC, we may calculate the highest w_I so as to put its contribution to w_T within the limits of this experimental error. First, we determine the upper limit of w_I, and thus the maximal injected volume, which is still acceptable with respect to experimental error. Further, we find out whether it is better to inject such a maximal solution volume at a minimal concentration at which the ratio of detector response to the noise of the base-line is sufficient, or a smaller solution volume at a higher concentration, and examine the role of concentration effects in this case.

Thus, for analytical SEC we have to determine if in the case of injection of a certain amount of polymer in solution acceptable with respect to the sensitivity of detection one has to prefer injection of a larger solution volume at a lower concentration, or vice versa. For preparative fractionation by means of SEC in those cases where the maximal capacity of the separation should be employed, one has to determine the upper limits of concentration and injected volume at which the influence of spreading caused by injection and of concentration effect on the results of fractionation is still acceptable. Similarly to the analytical SEC, in those cases where it is not necessary to employ the maximal capacity of the separation system, one has to determine optimal conditions with respect to the concentration and total volume of the injected polymer solution.

In order to express quantitatively the dependence of the elution volume V_e on concentration g_I and on the total amount of the injected polymer solution (which may be characterized by the standard deviation of the injection function σ_I), we employ theoretical relations derived in our earlier papers (1-3). At infinite dilution, the linear part of the dependence of the elution volume V_{eo}, on the hydrodynamic volume, $v \cdot \varepsilon_o$, of separated macromolecules may be described by an empirical equation

$$V_{eo} = P + Q \ln (v \cdot \varepsilon_o) \tag{2}$$

where P,Q are constants, v is the volume of an unswollen coil and ε_o is a dimensionless swelling factor at infinite dilution (4).The elution volume V_e depends on concentration according to a relation

$$\begin{aligned} V_e = P+Q\Big[\ln v &+ \frac{B^2 g_I^2 \sigma_I^2}{(\sigma_T^2-\sigma_I^2)A^2} \cdot \ln \left(\frac{\sigma_T A + B g_I \sigma_I}{\sigma_I A + B g_I \sigma_I}\right) - \\ &- \frac{B g_I \sigma_I}{(\sigma_T+\sigma_I)A} + \frac{\sigma_I^2}{\sigma_T^2-\sigma_I^2} \cdot \ln (A + B g_I) - \\ &- \frac{\sigma_T^2}{\sigma_T^2-\sigma_I^2} \cdot \ln\left(\frac{\sigma_T A + B g_I \sigma_I}{\sigma_T}\right)\Big] + \\ &+ k'\left(\frac{2[\eta] g_I \sigma_I}{\sigma_T+\sigma_I} + \frac{2\, k_H [\eta]^2 g_I^2 \sigma_I^2}{\sigma_T^2 - \sigma_I^2} \cdot \ln \frac{\sigma_T}{\sigma_I}\right) \end{aligned} \tag{3}$$

derived in our earlier papers (2,3) for a change in V_e with concentration due to a change in the effective dimensions of separated macromolecules and dynamic viscosity phenomena. In Eq.(3), A and B are constants defined by

$$A = \frac{1}{\varepsilon_o} , \quad B = \frac{1}{g_x}\left(\frac{\varepsilon_o - 1}{\varepsilon_o}\right) \tag{4}$$

where g_x is a critical concentration (4) at which dimensions of the macromolecules in solution are the same as under the theta thermodynamic conditions; $[\eta]$ is intrinsic viscosity, k_H is the Huggins constant and k' is a constant characterizing the given separation system with respect to the contribution of the dynamic viscosity phenomenon (1-3) and still having an empirical character. In addition to the mentioned contributions, i.e. to the change in the dimensions of separated macromolecules and viscosity phenomena, some complementary contributions may also be taken into account (1). Since the physical meaning and the absolute value of these contributions have not yet been explained unambiguously (5), they are not considered here. The above relations may be employed for a theoretical estimate of the effect of concentration, g_I, or of the injected volume of polymer solution characterized by σ_I on the elution volume V_e while maintaining the total constant amount of the injected sample characterized by the product $g_I \times \sigma_I$.

EXPERIMENTAL

All SEC measurements were performed using an apparatus built at this Institute. A differential refractometer R-403 (Waters Associates, Inc., Milford,Mass. USA) was used as the concentration detector. The separation columns were packed with silicagel Porasil (Waters), types B, D, E, and connected in a series consisting of two columns of each type in the order 2xE, 2xD, and 2xB. The whole separation column system was thermostated at 25± 0.5°C. Tetrahydrofuran distilled from cuprous chloride and potassium hydroxide was used as solvent, with the flow-rate 0.375 ml/min. The elution volumes were measured by using a siphon, 1.704 ml in volume. The separation system was calibrated by means of polystyrene (PS) standards (Waters). The calibration plot is shown in Fig. 1. Solutions of PS standards at concentrations of 0.05 % (w/v) were injected using a six-way injection valve (Waters) provided with a loop, 1.636 ml in volume. The effect of the ratio of concentration to the injected volume at a total constant injected amount of the polymer was determined with a PS standard, molecular weight M_w= 694 000 (Knauer, Oberursel, FRG). The injected concentrations in this case were 0.2, 2, and 4% (w/v), the corresponding injected volumes were 1.25, 0.125, and 0.0625 ml, respectively. These volumes of solutions of the PS standard were injected with the loop switched into a hydraulic circuit for 200, 20, and 10 sec. Hence, the total injected amount of the PS standard was 2.5 mg in all cases.

RESULTS AND DISCUSSION

The attainable reproducibility of elution volumes in liquid chromatography, and hence in SEC, lies approximately in the limits ± 0.1%. This means that if the width of the injection w_I is to affect the resulting w_T only within the limits comparable with experimental errors of the elution volumes, w_T/w_{To} must be equal to the maximal value 1.001. w_{To} is a hypothetical width of the elution curve for a case where $w_I = 0$. With respect to Eq.(1) it holds that

$$\frac{w_I^2}{w_{To}^2} + 1 = (1.001)^2 \qquad (5)$$

Since it holds approximately that $w_T \approx w_{To}$, we obtain after the rearrangement of Eq.(5)

$$\frac{w_I}{w_T} = \sqrt{(1.001)^2 - 1} = 0.0447 \qquad (6)$$

Hence, under the given conditions the maximal injection width characterized by w_I is approximately 4.5% of w_T.

In a theoretical investigation of the effect of the ratio of concentration g_I to the injected solution volume characterized by the standard deviation of the injection function σ_I, Eq.(3) was employed. The total weight amount of the injected polymer was constant. The model calculations were performed with the experimental parameters of Eq.(3) from our earlier paper (3). These values are summarized in Table 1. The separation system used in this paper was designed in order to cover a wide range of molecular weights of the polymers (cf. calibration plot in Fig.1), and has already been employed because of its high efficiency in the determination of molecular weight distribution of many polymers. At the same time, such a system is most frequently used in many laboratories. In our case we had no suitable polymer standard with narrow molecular weight distribution and with a sufficiently high molecular weight which would allow us to determine reliably the constant k' necessary for the calculation of V_e using Eq.(3). This empirical constant is determined by measuring the concentration dependence of V_e for a totally excluded polymer sample (2). The results of model calculations are summarized in Table 2, which also gives changes (in per cent) of the elution volumes ΔV_e due to the particular contributing processes, as well as the total change (again in

TABLE 1

Values of the parameters used in the calculation of the elution volumes from Eq.(3) of the PS standard with M_w=867 000

P	Q	$vx10^{17}$	A	B	σ_T	k´	$[\eta]$(ml/g)	k_H
-85.89	-3.01	8.855	0.357	75.48	1.75	7.957	211.71	0.362

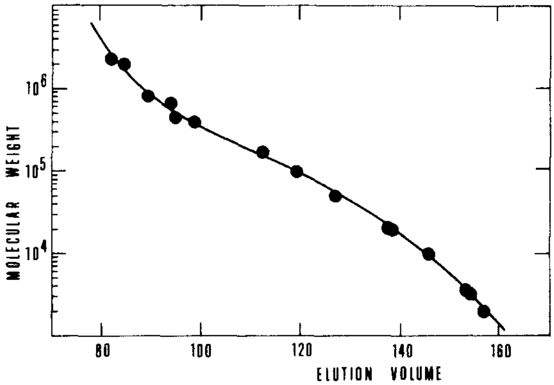

FIGURE 1. Calibration plot of the column separation system

per cent) of the elution volume ΔV_e caused by a change in g_I/σ_I at a constant $g_I x \sigma_I$. In the first set of ΔV_e values in Table 2, the value of $g_I x\ \sigma_I = 0.05$; up to $\sigma_I = 0.05$ the requirement that σ_I should be approximately 4.5% of $\sigma_T = 1.75$ is fulfilled. It can be seen that within the whole range $0.001 \leq \sigma_I \leq 0.1$ or in the respective concentration range covering two orders of magnitude, $0.5\% \leq g_I \leq 50\%$ (w/v), the total change ΔV_e and the particular changes corresponding to the contributing processes are almost constant; ΔV_e slightly decreases from approximately 6.5%

TABLE 2

Change in the elution volume caused by the injection of a solution of the PS standard of the given concentration and volume with respect to zero concentration or volume expressed in per cent

g_I(%)	σ_I	ΔV_e (%)		
		Change in effective dimensions	Viscosity phenomena	Total
50	0.001	1.470	5.025	6.495
10	0.005	1.470	4.863	6.333
5	0.01	1.467	4.787	6.254
2	0.025	1.461	4.666	6.127
1	0.05	1.452	4.540	5.992
0.5	0.1	1.427	4.361	5.788
0.1	0.5	1.210	3.489	4.699
10	0.025	5.839	31.367	37.206
5	0.05	5.818	29.437	35.255
2	0.125	5.733	26.451	32.184
1	0.25	5.539	23.617	29.156
0.5	0.5	5.127	20.038	25.165

to approximately 5.8% with concentrations ranging between 50% and 0.5% (w/v). Only if σ_I distincly exceeds the limit of 4.5% from σ_T, i.e. for $\sigma_I = 0.1$ and 0.5 at $\sigma_T = 1.75$, and if the concentrations are lower than 0.5% (w/v) the decrease in ΔV_e with concentration is distincly steeper. In the second set of ΔV_e values in Table 2, $g_I x \sigma_I = 0.25$, which is five times higher than in the preceding case. The ΔV_e values expressed in per cent are therefore also correspondingly higher. In this case the dependence of ΔV_e on concentration is evidently steeper than in the preceding case, even though, e.g., an increase in concentration from $g_I = 5\%$ to 10% (w/v) causes a change in ΔV_e from c. 35% to c. 37%, i.e. a relatively small change. Similarly to the preceding case, a steeper decrease in this dependence occurs in the range of low concentrations or of high σ_I. Of course, the σ_I values in the second set are higher in most cases than 4.5% from σ_T. It may be inferred from the results of the theoretical calculation that a rise in concentration compensated by a simultaneous decrease in the injected volume of polymer solution (with the same total injected wight amount of the polymer) leads to an increase in the elution volume. However this change in most of the practical applications is almost comparable with experimental errors,and hence almost negligible, providing that σ_I compared with σ_T lies within the limits determined in advance with respect to the required precision of fractionation.

The results of an experimental study of the effect of the ratio of concentration to the injected volume of the PS standard at a constant total weight amount of the injected polymer are summarized in Table 3. The experimental procedure has been desribed in the Experimental. The concentration range exceed one order of magnitude from 0.2% (w/v) to 4% (w/v), with the respective injected volumes of the solution of the PS standard 1.25 to 0.0625 ml. As documented by the results in Table 3, the elution volume V_e remained the same within the limits of experimental error in all these cases. A similar experimental finding has been described by Moore (6). Also, the polydispersity M_w/M_n of this standard calculated from direct experimental data without corrections is virtually unaffected by variation of the experimental conditions of injection. For the sake of comparison, we also injected 1.25 ml of a solution of the PS standard at the concentration 2% (w/v), i.e. a tenfold weight amount of the polymer (25 mg). The resulting elution volume is higher by more than 9% compared with the preceding measurements. The experimental results confirmed

TABLE 3

Effect of the injected volume and concentration on the elution volume of the PS standard with M_w=694 000

Concentration of injected solution (% w/v)	Injected volume (ml)	V_e	M_w/M_n
0.2	1.25	94.5	1.10
2.0	0.125	94.6	1.12
4.0	0.0625	94.3	1.13
2.0	1.25	103.5	1.19

excellent agreement with the preceding theoretical calculations. They also demonstrated that in the range where the width of injection within the limits of experimental error is negligible, (compared with the width of the elution curve), the injection conditions, i.e. concentration and the total volume of injected solution, may **be** broadly varied (if the total weight amount of the polymer remains constant), without any pronounced effect on the results of fractionation by the SEC method.

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