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On the Origins of Flow-Rate Dependence of Elution Volume in Gel Permeation Chromatography

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Abstract

The peak elution volume of a narrow polystyrene fraction in a porous silica column is a function of the solvent flow rate through the column. The explanation for this effect offered by Gudzinowicz and Alden and others is that the equilibrium assumption, usually made in size exclusion chromatography, is no longer valid at high flow rates. An alternative explanation based on a flow-rate-dependent equilibrium distribution coefficient (ratio of intrapore polymer concentration to extrapore polymer concentration) is offered here. This view is supported by experimental results for the equilibrium flow-rate-dependent column retention measured previously in these columns.

INTRODUCTION

Several groups of workers (*1–4*) have reported that the peak elution volumes of monodisperse polymer samples in size exclusion chromatographic columns depend on the flow rate of the mobile phase through the column. Classical chromatographic theory (*5*) shows that when thermodynamic equilibrium is achieved at every point within the column, the elution volumes depend only on the nature of this equilibrium and *not* on the flow rate through the column. Thus observations of flow-rate-dependent elution volumes have been attributed to nonequilibrium effects (*1*, *2*). In other words, macromolecular diffusion between the mobile and stationary phase is sufficiently slow so that equilibrium cannot be attained at each point in the column.

Our recent work on the behavior of macromolecules in complex velocity fields, and in flow through packed porous chromatographic columns in particular, has led us to the conclusion that flow-rate-dependent peak elution volumes may also arise from a somewhat different physical basis. The purpose of this article is to communicate the observations and considerations which have led us to these conclusions.

The flow field through packed columns belongs to a class of flows referred to as kinematically nonhomogeneous, meaning that the strength of the velocity gradient varies from point to point within the flow field. Macromolecules are subject to gross configurational distortion depending on the strength of the local velocity gradient which they experience. Several authors (*6–8*) have speculated that, in nonhomogeneous flows, the lowest velocity gradient regions of the flow can become enriched in polymer concentration as the macromolecules attempt to minimize configurational entropy by escaping the elongating and orienting effects of the high velocity gradient regions. The quantitative aspects of this speculation have been explored (*9*). No direct measurements of this effect exist, although many experiments indirectly support the idea (*10*). We have recently shown (*11*) that in *any* nonhomogeneous flow, macromolecules do not move with the local solvent velocity, leading again to the possibility of a nonuniform spatial concentration distribution. Purely hydrodynamic arguments are sufficient to show this, with no *ad hoc* thermodynamic assumptions as above. Cross-streamline migration is predicted toward the concave side when the local velocity field is curvilinear and nonhomogeneous. Clearly, the opportunity for both these modes of migration, thermodynamic and hydrodynamic, exists in principle in the flow through chromatographic columns. Maximum velocity gradient regions, of magnitude 100 to 1000 $\sec^{-1}$ under reasonable operating conditions, exist in close proximity to stagnant, low velocity gradient regions. Macromolecules also travel curved paths in circumnavigating the packing particles. Thus it is likely that nonuniform concentration profiles may arise from the fluid mechanics in chromatographic columns.

In a set of experiments designed to determine if such nonuniform concentration profiles actually develop, we have recently studied (*12*) the continuous flow of dilute polymer solutions through size exclusion chromatographic (SEC) columns. These experiments involved flowing dilute polymer solutions through the columns continuously and making step changes in the flow rate (with constant inlet concentration). By monitoring the column effluent concentration continuously, the change in column retention could be monitored as a function of flow rate. Figure 1 is an example of the changed effluent concentration for flow rate changes of first 3 to 1 mL/min and then back to 3 mL/min with polystyrene in tetrahydrofuran in an SEC column of nominal pore size 1000 Å. The effluent concentration is seen to be higher than the steady-state inlet–outlet concentration for a short period of time when the flow rate is decreased,

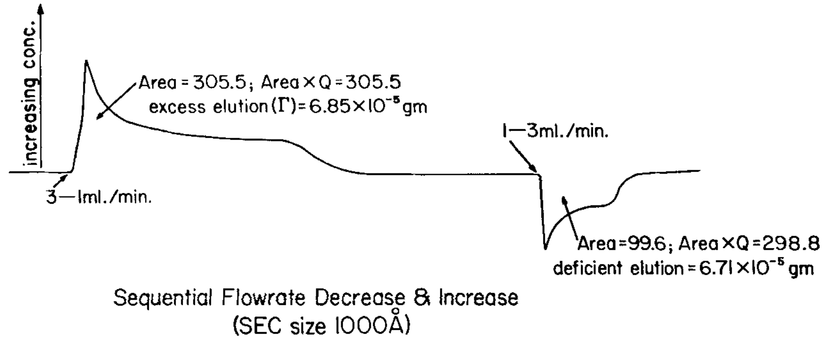

FIG. 1. Typical detector responses to step flow rate changes (3 to 1 mL/min, then 1 to 3 mL/min) for PS ($\bar{M}_n = 1.8 \times 10^6$) in THF in the 1000 Å SEC column.

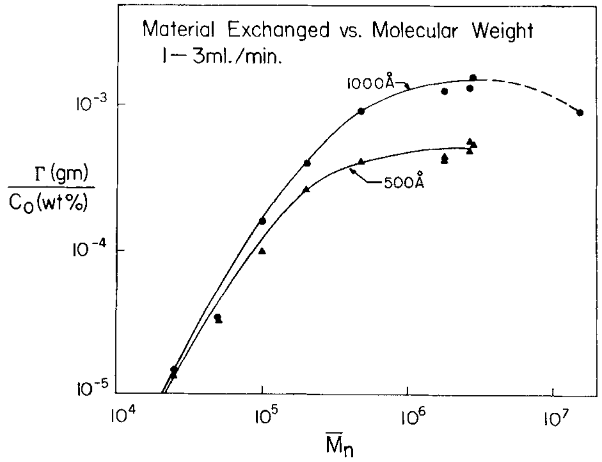

FIG. 2. Summary of experimental results on the flow-rate-dependent polymer retention as a function of PS molecular weight and SEC pore size.

and the opposite is seen when the flow rate is increased. This indicates that the column retention decreases when the flow rate is decreased, and vice versa.

The change in column retention can be calculated from

$$\Gamma = \int_0^\infty \Delta C(t) Q_2 \, dt \qquad (1)$$

In this equation Γ is the changed column retention, $\Delta C(t)$ is the difference between the effluent concentration and the steady-state inlet concentration

C_0, and Q_2 is the final flow rate setting. Figure 2 is a summary of all of the measurements previously (*12*) taken on the flow-rate-dependent column retention as a function of polymer molecular weight and SEC pore size. The relation between these experimental results and the macromolecular migration theories is discussed elsewhere (*12*). We propose to use these experimental results to give an alternative interpretation for flow-rate-dependent peak elution volumes.

It is expected that the observed flow-rate-dependent retention in the SEC columns should have a significant effect on the resolution characteristics of these columns. In fact, the changing column retention indicates that the apparent distribution coefficient between stagnant micropores and interstitial areas is a function of flow rate. The usual equilibrium theory of size separations relates the elution volume of a species to that species' distribution coefficient. If the distribution coefficient depends on flow rate, so then does the elution volume. We believe that this changing distribution coefficient may arise from the nonhomogeneous flow effects described earlier.

EXPERIMENTAL

Apparatus

The experimental apparatus consisted of a DuPont 830 high pressure liquid chromatograph. A DuPont porous silica column with a narrowly distributed pore size of 1000 Å was used. A DuPont 833 flow controller maintained the flow rate through the column within ±0.5% of the setting. The effluent from the columns was monitored continuously with a UV detector employing monochromatic light of wavelength 254 nm. Samples were injected into the top of the column through a six-port injection valve system.

Procedure

Dilute polystyrene (PS) solutions (0.05 wt-%) in tetrahydrofuran (THF) were injected into the column at a number of flow rates, and the peak elution volumes were measured. Narrow molecular weight distribution polystyrenes obtained from Pressure Chemical Co. were used. The PS molecular weights and polydispersities are summarized in Table 1. The flow rates through the column were calibrated by collecting the effluent stream over a long period of time in a graduated cylinder. A close contact was maintained between the graduated cylinder and the instrument tubing to minimize solvent evaporation during the calibration. The accuracy of the

TABLE 1

Polystyrene Standards Used from Pressure Chemical Co. and Their Polydispersity

Molecular weight $\bar{M}_n$ (g/g-mole)	Polydispersity $\bar{M}_w/\bar{M}_n$
5.0×10^4	<1.06
2.0×10^5	<1.06
3.9×10^5	<1.10
9.0×10^5	<1.10
2.85×10^6	<1.30

TABLE 2

Measured and Predicted Dependence of Elution Volume on Flow rate in the DuPont Silica Columns

	Measured quantities				Predicted quantities	
	Elution volume (mL)					
$\bar{M}_n$	$Q = 1$	$Q = 3$	K_D, $Q = 1$	Γ/C_0, 1 to 3 mL/min	K_D, $Q = 3$	Elution volume, $Q = 3$ mL/min
2.85×10^6	3.57	3.90	0.022	1.67×10^{-3}	0.076	3.74
9.0×10^5	3.82	4.02	0.102	1.20×10^{-3}	0.141	3.94
3.9×10^5	4.35	4.47	0.272	7.80×10^{-4}	0.297	4.43
2.0×10^5	4.85	4.92	0.431	4.00×10^{-4}	0.444	4.89
5.0×10^4	5.60	5.67	0.671	5.30×10^{-5}	0.673	5.61
Benzene	6.58	6.63	1.0	0.0	1.0	6.58

calibration was substantiated by the equivalent elution volumes measured for benzene at various flow rates.

Results

Table 2 is a summary of the measured elution volumes for PS in THF in the 1000-Å column at flow rates of 1 and 3 mL/min. Figure 3 is the calibration plot of the 1000 Å column for PS in THF at a flow rate of 1 mL/min. From Table 2 it is seen that the elution volumes and the column calibration are functions of the flow rate through the column.

DISCUSSION

The experimental results show that the elution volume of various molecular weight polystyrenes in the SEC column increases with increasing solvent flow rate. The flow-rate dependence of the elution volume also

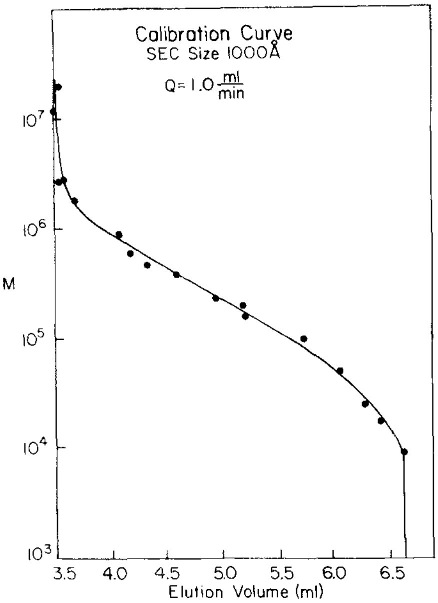

FIG. 3. Calibration curve for the 1000 Å SEC column with PS in THF at a flow rate of 1 mL/min.

increases greatly with molecular weight. These data are in agreement with that of Gudzinowicz and Alden (*2*). They observed the elution volume of PS increase with the flow rate in similar glass bead columns.

The basic equilibrium approach to SEC is to relate the elution volume of a macromolecular species to a distribution coefficient for that species:

$$V_e = V_0 + K_D V_I \tag{2}$$

where V_e is the peak elution volume, V_0 is the fluid volume of the column external to the pores, and V_I is the intrapore fluid volume of the column. The distribution coefficient is K_D, defined as the ratio of the intrapore polymer concentration to the extrapore polymer concentration at equilibrium. The basic assumption of the equilibrium theory is that there is sufficient time for equilibrium to be established at all points and times in the column as the polymer solution is eluted through it. As the flow rate is increased, a point is eventually reached at which equilibrium is no longer maintained.

The total column fluid volume for the 1000 Å-column, V_T (intrapore and extrapore), can be obtained from the elution volume of benzene ($K_D = 1$). The calibration plot (Fig. 3) shows V_T to be 6.63 mL. The extrapore column fluid volume, V_o, can be obtained from the elution volume of a very high, excluded, molecular weight species ($K_D = 0$). The calibration plot shows V_o to be 3.5 mL. The intrapore fluid volume of the column is then just the difference between these, or 3.13 mL. The apparent distribution coefficient can be obtained for each molecular weight shown in Table 1 at a flow rate of 1 mL/min from

$$V_e = 3.5\,\text{mL} + K_D(3.13\,\text{mL}) \tag{3}$$

Gudzinowicz and Alden (*2*) attribute the increasing elution volume with flow rate to the fact that equilibrium is no longer maintained at higher flow rates, and therefore Eq. (2) is no longer applicable. An alternative explanation is offered here. It is suggested that elution volume increases with flow rate because the distribution coefficient increases with flow rate.

A flow-rate-dependent distribution coefficient has been experimentally observed in flow-rate-dependent polymer retention studies (*12*). The results of this study are summarized in Fig. 2. These experimental results can be used to predict the change in distribution coefficient with a change in solvent flow rate from

$$K_{D(Q_2)} = K_{D(Q_1)} + \frac{(\Gamma/C_0)_{Q1-Q2}}{V_I} \tag{4}$$

The quantity $(\Gamma/C_o)_{Q1-Q2}$ is equal to the changed column retention for a flow rate change from Q_1 to Q_2 divided by the inlet polymer concentration C_0. With this equation the elution volume at a higher flow rate, Q_2, can be calculated from Eq. (2). In Table 2 are the experimentally measured elution volumes at flow rates of 1 and 3 mL/min, and also the predicted elution volumes using the measured distribution coefficient at 1 mL/min, the experimental results shown in Fig. 2, and Eq. (4).

The agreement between the measured and the predicted increase in elution volume for flow rates of 1 and 3 mL/min is close. The elution volumes actually increase more than the predicted increase.

CONCLUSIONS

The experimental data reported here and previously (*12*, *13*) show that the elution volume increases with the flow rate to a large extent because the distribution coefficient increases with the flow rate. Even if complete equilibrium is maintained at the higher flow rate, the elution volume will still increase with the flow rate. The fact that the elution volume increased even more than predicted by the fluid mechanically modified equilibrium theory indicates that part of the increase was due to nonequilibrium effects. We believe the origins of this effect are in the interactions between the fluid mechanics and the macromolecular shape during flow through the column. As outlined elsewhere, these interactions can produce nonuniform spatial concentration distributions in the column and hence a flow-rate-dependent distribution coefficient. We are continuing our experimental and theoretical studies of this effect.

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