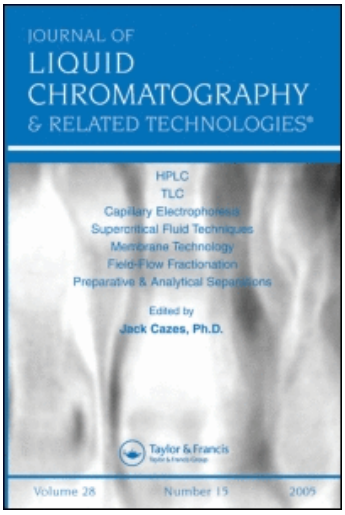

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Flow Rate Dependence of Elution Volumes in Size Exclusion Chromatography: A Review

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To cite this Article Aubert, J. H. and Tirrell, M.(1983) 'Flow Rate Dependence of Elution Volumes in Size Exclusion Chromatography: A Review', Journal of Liquid Chromatography & Related Technologies, 6: 9, 219 — 249
To link to this Article: DOI: 10.1080/01483918308062875
URL: http://dx.doi.org/10.1080/01483918308062875

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(S-2), 219-249 (1983)

Flow Rate Dependence of Elution Volumes in Size Exclusion Chromatography: A Review

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Abstract

This review summarizes experimental data which indicate that elution volumes can change with flow rate in size exclusion chromatography experiments. The mechanisms resulting in flow rate dependent elution volumes are discussed. They can be roughly divided into two classes: 1) anomalous effects, and 2) flow rate dependence of the partitioning of molecules into the micropores of the column packing. The partitioning can depend on flow rate if the partition coefficient is concentration dependent, if viscous fingering occurs, or if molecular migration phenomena are important.

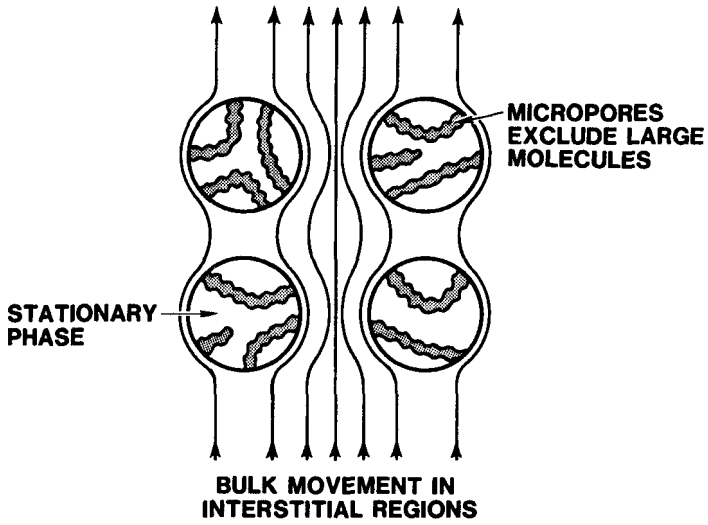
0148-3919/83/06S2-0219$3.50/0

1. Introduction

Size exclusion chromatography (SEC) is a widely used experimental technique which separates macromolecules on the basis of size. The technique is often used to determine the molecular weight distribution of synthetic and natural polymers. Size exclusion chromatogaphy has also been called gel permeation chromatography (GPC) or gel filtration for aqueous solutions; however, size exclusion is the most appropriate name because separation occurs as a result of larger molecules being excluded from the stagnant pores of a chromatographic column to a greater extent than smaller molecules.

In a chromatographic experiment, solvent is pumped continuously through the SEC column. At the desired time, a small sample of a macromolecular solution is injected at the top of the column as a pulse. As the macromolecules flow past the micropores, which are inside of the column packing particles, they partition between the micropores and the interstitial volume, (Fig. 1), i.e., between the stationary and the mobile phases. The partitioning depends upon the pore size and type and the macromolecular size and type. In the absence of adsorption, larger molecules are excluded from the pores to a greater extent than smaller molecules. Since the micropores are relatively stagnant, and the interstitial volume is not, larger molecules move through the column faster than smaller molecules and elute first. Hence, an SEC column separates macromolecules on the basis of size.

The first applications of SEC occurred in biochemistry, where biological macromolecules were separated. Porath[1] used dextran gels in a chromatographic column and separated proteins, peptides, amino acids, and some of their derivatives.



(1) The transport of macromolecules through an SEC column is dependent on the partitioning into the micropores of the column packing.

Based upon the experimental results, Porath speculated that the mechanism of separation was the exclusion of large molecules from the gel. Shortly thereafter, Porath[2] proposed a theory of SEC based upon equilibrium partitioning of macromolecules between unbounded solutions and conical pores. Good qualitative agreement was found between the predictions of this theory and experimental data on low molecular weight dextran fractions obtained by Granath and Flodin.[3] More detailed theoretical descriptions of the partitioning in SEC followed. Squire[4] extended the theoretical development by determining the partitioning of spherical macromolecules in gels modeled as a combination of cones, cylinders, and crevices. Laurent and

Killander[5] determined the partition coefficient of spherical macromolecules in gels modeled as a three-dimensional network of fibers. These theories made SEC nearly unique among types of chromatography, since its behavior could be predicted reasonably accurately.

Moore[6] was the first to apply SEC to synthetic polymers, by separating polystyrenes of narrow molecular weight fractions in a chromatographic column packed with beads, which were made by crosslinkng a polystyrene gel in the presence of diluents. The fractions were shown to be efficiently separated, and this demonstrated the usefulness of SEC in characterizing synthetic polymers in order to aid in their manufacture and use. However, the fact that SEC is the useful technique it is today owes much to the work of Grubisic, Rempp, and Benoit.[7]

In order to use SEC to determine the molecular weight distribution of an unknown sample, one must calibrate the SEC columns used. Calibration consists of determining the elution volumes for polymers of known molecular weight and is usually compiled as a plot of the logarithm of molecular weight versus elution volume. By measuring the elution volume of the unknown sample, its molecular weight can be determined by comparison to the calibration curve. This type of calibration depends upon the polymer/solvent system used in the columns. Grubisic, Rempp and Benoit[7] discovered a nearly universal calibration method for SEC; i.e., a calibration technique for an SEC column which is independent of the solvent or polymer used. A plot of the logarithm of the intrinsic viscosity and molecular weight product ([n]M), versus elution volume, for a particular column, was found to yield a characteristic curve which was independent of the polymer/solvent system. They

deduced that the important macromolecular size in SEC is the hydrodynamic volume, since the intrinsic viscosity and molecular weight product is proportional to this.

Theoretical explanations for the above followed in the work of Casassa,[8,9] Casassa and Tagami,[10] and Giddings et al.[11] These workers calculated the equilibrium partition coefficients for macromolecular models partitioning between an unbounded solution and micropores of simple geometric shape. If the elution of a macromolecular solution through a chromatographic column is sufficiently slow so that equilibrium is maintained between the solute in the flowing stream adjacent to the pore mouth and all of the pore volume, then these theoretical results could be used to predict the elution characteristics of a macromolecule of known size and structure. The relationship between the elution volume and the equilibrium partition coefficient is predicted to be[12]

$$V_e = V_O + K_D V_I \ , \qquad (1)$$

where V_e is the measured elution volume for the macromolecule, V_I is the total volume of the micropores, V_O is the interstitial volume in the column, and K_D is the equilibrium partition coefficient. If equilibrium is not maintained between the solution near a pore mouth and the entire pore volume, then diffusion of the macromolecules could be important. The significance of macromolecular diffusion in SEC was not thoroughly understood until the work of Hermans.[13]

If the equilibrium assumption in SEC is valid, then the elution volume should be independent of flow rate, as predicted

by Eq. (1). The majority of earlier workers concluded that elution volmes were independent of flow rate within the usual flow rate ranges.[14] However, a number of studies have shown that elution volumes can either increase or decrease with flow rate, depending upon the experimental conditions.[15-21] Some of these observations have been rationalized, but others remain unexplained. Some of the possible explanations for these observations of flow rate dependent elution volumes include nonequilibrium effects, molecular structure changes with flow rate, stationary phase changes with flow rate, concentration/flow rate effects, instrumental anomalies, and molecular migration effects. In this review we will summarize, in a mechanistic manner, experimental data which indicate a flow rate dependence of elution volume and some thoughts on the most likely causes for each of the observations.

2. Nonequilibrium Effects

Nonequilibrium effects in SEC can occur in two ways. The first is when equilibrium is not maintained at the micropore mouth between the solution in the interstitial volume and the solution within the micropore. The second is when equilibrium is maintained at the micropore mouth but the solution in the interior of the micropore is not at equilibrium with the solution in the interstitial volume. In this case diffusion of the solute into the micropore could be important. Nonequilibrium effects have been used by a number of authors[15,17,26,27] to explain flow rate dependent elution volumes in SEC.

2.1. Hermans' Model of SEC

Hermans[13] proposed a mathematical model of the chromatographic process in order to understand and assess the role of

diffusion in SEC. Hermans assumed the following about the column and packing:

i) The packing consists of rigid spherical particles with a uniform diameter. Most modern SEC packings are very uniform and many consist of spherical particles.

ii) The column is spatially homogeneous; hence, in any axial cross section of the column the concentration of solute is independent of angular or radial position. Most SEC columns are packed carefully to insure that this assumption is satisfied, since the column resolution diminishes if it is not. Also, the flow through the column is assumed to be plug flow, with no angular, radial, or axial variation in the flow rate.

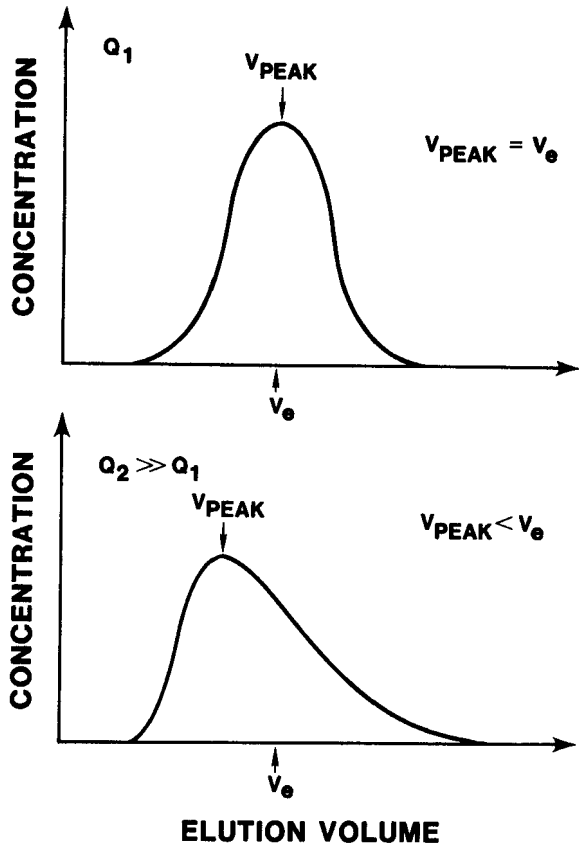
iii) No dispersion of the solute occurs in the mobile phase due to diffusion; i.e., convection dominates the transport of solute in the mobile phase. At the flow rates typically used in SEC, this assumption is valid, since the diffusion coefficients of high molecular weight species are extremely low.

iv) Equilibrium between the solute concentration at the packing surface (but not within the packing particle) and the solute concentration in the mobile phase adjacent to each packing particle is maintained at all times. This assumption has been experimentally verified by a number of independent workers[22-24] whose work is summarized below.

The important diffusion process in the model occurs in the transport of solute from the surface of the packing particles to the interior of the particles.

Hermans solved the mathematical problem with Laplace transforms and obtained exact expressions for the moments (in time) of the solute concentration leaving the end of the column. For the first moment (the mean) an expression identical to Eq. (1) is obtained. This is an extremely important result. Even in the absence of equilibrium between the mobile phase solute concentration and the solute concentration in the pore volume, Eq. (1) predicts the mean elution volume of the solute if equilibrium is maintained at the packing surface. Hence, the theoretical results obtained by Casassa,[8,9] Casassa and Tagami,[10], and Giddings et al.[11] can be used to predict the elution volume of a macromolecular solute, even when diffusion into the packing is slow enough to be significant. Although the first moment is independent of the diffusion of solute into the pores, higher moments do depend on the diffusion rate. Therefore, the peak width and the shape of the elution curve depend on the flow rate through the column and the diffusion of the solute.[5,12,13]

At low flow rates the elution curve is Gaussian in shape, and therefore symmetrical, and the peak position is equal to the first moment. At higher flow rates, however, the elution curve can become skewed when the diffusion rate into the micropores is sufficiently slow,[13] (Fig. 2). If the first moment is independent of flow rate, as predicted by Hermans' theory, and the elution curve becomes more skewed with increasing flow rate, then the peak position of the elution curve should decrease as the flow rate increases. Hence, when observing flow rate dependent elution volumes, one must be careful to measure the position of the first moment, rather than the peak position. This may be an explanation as to why some

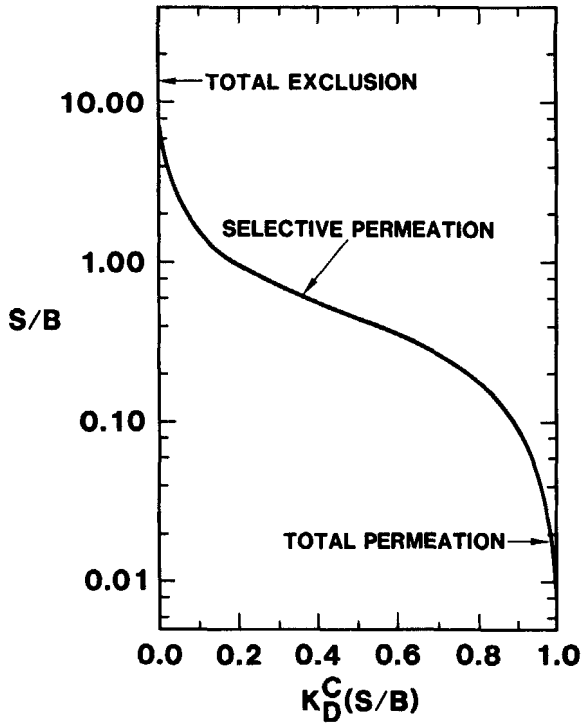


(2) The qualitative effect of flow rate on the skewing of an elution curve and the peak position.

experimentalists have observed elution peaks decreasing with increasing flow rates, even after other corrections had been made to the elution curve.(15)

2.2. Predicted Elution Curves

With the results of Hermans, the elution characteristics of a macromolecular solute can be predicted from a molecular theory for the equilibrium partition coefficient of



(3) SEC calibration curve predicted for the elastic dumbbell model in a chromatographic column. The chromatographic column is assumed to consist of a stationary phase containing uniform capillary micropores.

that macromolecule. Only the weak assumption, that equilibrium is maintained at the surface of the stationary phase, is necessary. One example of this is shown in Fig. 3, in which the macromolecules are modeled with the Rouse model and the micropores are approximated as capillaries.[25] The predicted equilibrium partition coefficient, K_D, is plotted against the ratio of the radius of gyration of the macromolecule to the capillary pore radius, (S/B). This calibration plot is

similar to the universal calibration proposed by Grubisic, Rempp, and Benoit[7] since K_D is linearly related to the elution volume in a chromatographic column, Eq. (1), and the logarithm of the radius of gyration is approximately proportional to the logarithm of the hydrodynamic volume. The similarity in shape of predicted calibration curves to experimentally observed ones gives support to the assumption that equilibrium is maintained at the surface of the stationary phase.

2.3. Comparison with Experiment

Although the shape of experimental SEC calibration curves is the same as that predicted theoretically with any of the equilibrium molecular theories[8-11,25] some questions have remained as to whether equilibrium is always maintained at the stationary phase surface at all flow rates and operating conditions. At high enough flow rates equilibrium may no longer exist, and, in fact, a number of authors have attributed flow rate dependent elution volumes to nonequilibrium effects.[15,17,26,27]

The importance of nonequilibrium at the packing particle surfaces can be estimated by comparing two time scales. The first is the time necessary for a macromolecule to enter a micropore in the stationary phase (or to equilibrate with the pore mouth), and the second is the time for the solute band to pass a micropore. Since the size of the micropores is comparable to the macromolecular size, the first time is essentially the primary relaxation time of the macromolecule, τ. The second time is relted to the flow rate, Q, and the column's loading or sample size, V_ℓ.

$$t = V_\ell / Q$$

Typical values of these parameters in a modern chromatographic experiment are Q = 1 cm^3/min and V_l = 50 μl, which result in 3 seconds as an estimate of the time for the solute band to pass any point in the column. Even a very high molecular weight polymer has a relaxation time at least four orders of magnitude smaller than this estimate of τ, and lower molecular weight species have even a smaller relaxation time. Hence, the ratio τ/t is so small that nonequilibrium effects should never be importnt in SEC. Van Kreveld and Van Den Hoed[28] arrived at this same conclusion in a more rigorous manner.

Yau, Malone, and Fleming [22] compared SEC partition coefficients, (obtained from Eq. (1) in a chromatographic experiment), to equilibrium partition coefficients for polystyrene in chloroform partitioning into both a porous glass packing and a polystyrene gel packing. They concluded that equilibrium was attained at the stationary phase surface in all of their experiments, which involved a range of flow rates. The same conclusion was reached in a similar study by Grubisic-Gallot and Benoit[23] and later by Aubert and Tirrell.[24] Hence, we can conclude that flow rate dependent elution volumes, although experimentally observed at times, are not due to nonequilibrium effects.

3. Molecular Structure Changes with Flow Rate

3.1 Molecular Degradation

A number of experimentalists have demonstrated that high molecular weight molecules can be degraded in a chromatographic column at high flow rates.[29-32] These studies have involved a number of different polymers and a number of different instruments. If high molecular weight species are degraded

into lower molecular weight species during flow through a chromatographic column, then their elution volume will increase. Hence, molecular degradation with increasing flow rate results in an apparent increase in elution volumes with increasing flow rates.

3.3. Configurational Changes

When macromolecules are subject to large rates of deformation, their configurations can change by stretching and aligning with the flow streamlines.[33] Such a situation can occur at sufficiently high flow rates in a chromatographic column. Some workers have suggested that this could change the partition coefficient of the macromolecule and, therefore, its elution volume could change with flow rate. These workers have concluded, however, that this effect is of no significance in SEC.[16] This observation is corroborated by molecular theories,[34] which show that the dilute solution partition coefficient of a macromolecule depends only upon the number density of macromolecules outside of the pore, the environment inside of the pore, and on equilibrium being maintained at the pore mouth.

4. Stationary Phase Changes with Flow Rate

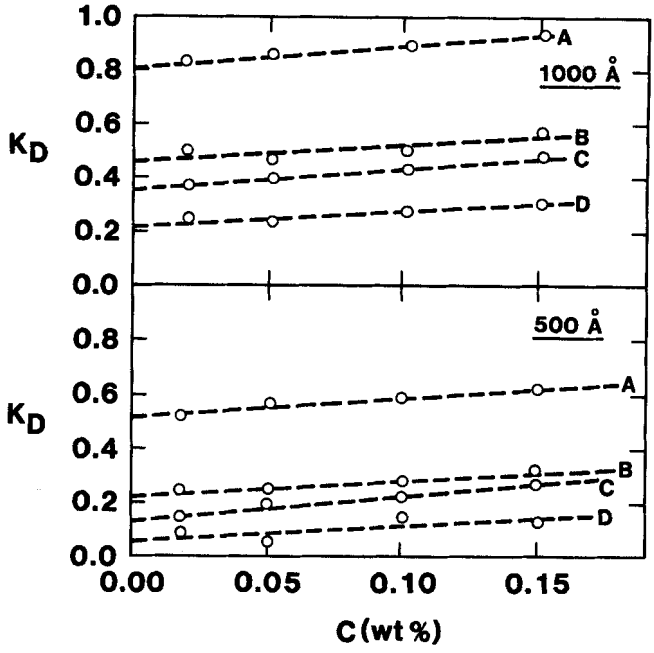
A possibility exists that flow rate dependent elution volumes could arise from changes in the pore volume or pore size with a change in flow rate and a corresponding change in the column's pressure drop. Either of these effects would result in the elution volume decreasing as the flow rate increases. This is a possible mechanism of flow rate dependent elution volumes in columns packed with nonrigid packings,[17] although most authors have concluded that this is not a significant effect in the range of flow rates and pressure drops typically used.[15]

Modern, high-pressure SEC exclusively uses rigid packings which are not affected by variations in the pressure drop. This has been experimentally verified for one rigid commercially available column packing.[35]

5. Concentration/Flow Rate Effects

Elution volumes have been observed to increase as the solute concentration is increased[16-20,36] and also as the volume injected is increased.[16,17,20] These observations suggest that, if equilibrium is maintained at the pore mouths, then the equilibrium partition coefficient must increase with increasing solute concentration. Such an effect has been experimentally shown to be true for both rigid and flexible macromolecules.

Brannon and Anderson[37] measured the equilibrium partition coefficients of three dextrans and bovine serum albumin partitioning into controlled-pore glass beads by a batch mass balance technique. The partition coefficients were measured as a function of concentration and, at low concentrations, were found to increase linearly with concentration. Satterfield et al.[38] had previously performed similar experiments on flexible polystyrene molecules and also found that the partition coefficients increased linearly with concentraton at low concentrations. Aubert and Tirrell[39] measured the partition coefficients of polystyre in a chromatographic packing as a function of concentration and molecular weight. These results are summarized in Fig. 4, and show the same linear dependence on concentration as does the elution volume in a chromatographic experiment[16-20,36] and also show the same qualitative dependence on molecular weight. A number of molecular theories



(4) Experimental partition coefficients as a function of concentration for two different pore sizes, 500Å and 1000Å. A: $\bar{M}_N = 1.1 \times 10^5$; B: $\bar{M}_N = 3.0 \times 10^5$; C: $\bar{M}_N = 9.0 \times 10^5$; D: $\bar{M}_N = 1.8 \times 10^6$.

predict that the partition coefficient should increase linearly with concentration at low concentrations.(40,41,24)

As the flow rate through a chromatographic column increases, the width of the elution peak increases,(12,13) and as a consequence the average concentration of the solute band decreases. Since equilibrium partition coefficients generally decrease as the solute concentration is decreased, the measured elution volume should decrease as the flow rate is increased, for samples of sufficiently high initial concentration.

Another concentration effect, one that is independent of the above, has to do with the viscosity of the injected sample. It has been shown that if the injected solution viscosity differs greatly from the solvent viscosity, then viscous fingering can occur in flow through the column. The amount of viscous fingering depends upon the flow rate and should decrease as the flow rate is increased. Hence, viscous fingering should result in elution volumes changing with flow rate.[16,17]

6. Instrumental Anomalies

Some experimentalists have shown that apparent flow rate dependent elution volumes can result from instrumental anomalies; i.e. the performance of the instrument depending upon the flow rate of solvent through it. Two such anomalies were documented by Yau, Suchan, and Malone[15] on instruments which use a siphon for the purpose of monitoring the flow and elution volume. The first anomaly was due to the fact that solvent would continue to flow into the siphon as it was discharging. This anomaly results in apparent elution volumes appearing lower than they actually are and in apparent elution volumes decreasing with increasing flow rate. The second anomaly was due to the fact that solvent could evaporate before the siphon discharged. At slow flow rates a significant portion of the solvent in the siphon could evaporate before the siphon discharged. This would also result in apparent elution volumes that were lower than the true elution volumes. However, since the evaporation effect decreases as the flow rate increases, the apparent elution volume would increase with increasing flow rate.

When both of these anomalies are operative, the apparent elution volume increases with flow rate at low flow rates and

then decreases with flow rate at high flow rates. A plot of apparent elution volume versus flow rate would show a maximum for a given molecular species. Yau, Suchan, and Malone[15] demonstrated that these two anomalies were the major contribution to their observed flow rate dependent elution volumes. With these instrumental anomalies accounted for, Little et al[42] found no flow rate dependence of elution volumes on an instrument that was similar to that used by Yau, Suchan, and Malone.

7. Molecular Migration Effects

Numerous studies have been done on the flow rate dependence of elution volumes in SEC utilizing nonrigid packings. The results of these studies have been very dependent upon the instrumentation, especially the column packing, and also upon the polymer/solvent system. Different authors have reported elution volumes increasing with flow rate, decreasing with flow rate, or being independent of flow rate.[14] Some of the results have been shown to be directly attributable to instrumental anomalies.[15,42] Other postulated causes of flow rate dependent elution volumes include dispersion effects and concentration/flow rate effects. Nonequilibrium has been shown to be of no significance in SEC. Modern SEC is run only at high pressure and usually only with rigid packings. In this section, some experimental results on flow rate dependent elution volumes occurring in columns with rigid packings are summarized. In addition one possible mechanism for this dependence is discussed.

Gudzinowicz and Alden[18] ran SEC experiments on narrow molecular weight distribution polystyrenes (PS) dissolved in tetrahydrofuran (THF) and measured the peak elution volumes at a number of flow rates. Their SEC column was packed with

44-50 μm diameter rigid, porous glass beads containing micropores of known sizes. Peak elution volumes were measured at a number of flow rates for various PS molecular weights. For low molecular weights (and in particular for benzene) there was little flow rate dependence of elution volumes. At higher molecular weights the flow rate dependence of the elution volume increased significantly. For the highest molecular weight studied ($\bar{M}_N$ = 1.8 x 10^6) the elution volume increased more than 8% as the flow rate doubled. Their results are summarized in Table I.

An experimental study of the flow rate dependence of elution volumes was also undertaken by Aubert and Tirrell,[21,24,35,43] who used a DuPont 830 high pressure liquid chromatograph. Narrow molecular weight distribution PS, obtained from Pressure Chemical Co., was used after dissolving in THF, (Table II). The column packing consisted of 6um diameter silica spheres with 1000A diameter micropores.[21,3] Figure 5 displays the superimposed elution peaks for some of the molecular weights studied at two different flow rates, 1 cm^3/min and 3 cm^3/min. It is clear that the peak elution volumes increased with increased flow rate. The peak widths also increased with flow rate, as expected from Hermans equilibrium theory.[13] For the highest molecular weight ($\bar{M}_N$ = 2.85 x 10^6) the first moment of the elutant peak could be calculated, since it was displaced far enough from the benzene peak so that no overlap of the peak bases occurred. The elution volume of the first moment increased slightly more with flow rate than the peak elution volume for this high molecular weight sample. The position of the first moment differed only slightly from the peak position since the skewing of the curves was minimal. At

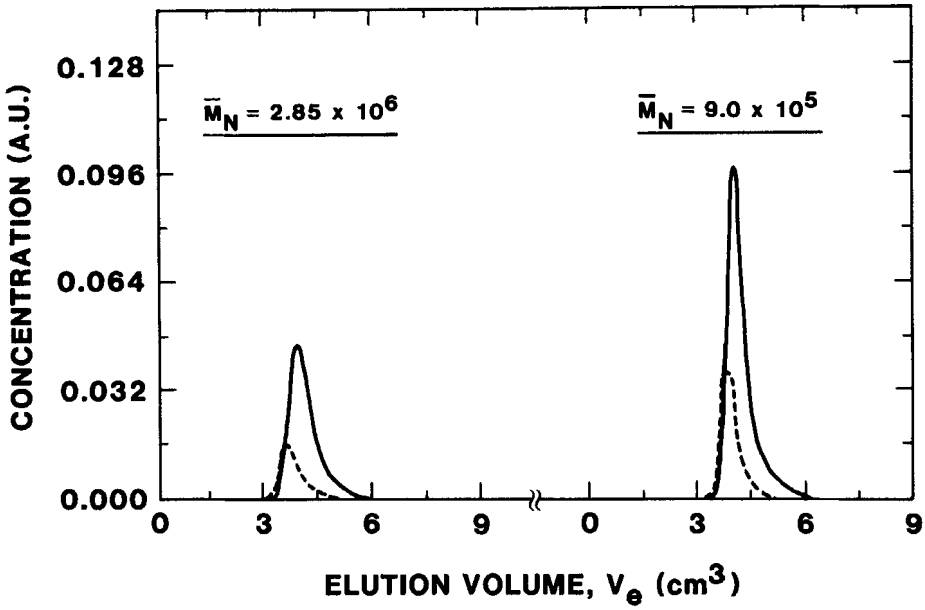
Table I Data of Gudzinowicz and Alden for flow rate dependent elution volumes for PS in THF in porous silica bead columns.

Run	Solvent Flowrate (ml/min)	$\bar{M}_N$	Elution Volumes			
		1.8×10^6	1.6×10^5	5.1×10^4	1.0×10^4	Benzene
A	0.728	12.32	15.50	17.83	20.10	22.33
B	0.907	12.53	15.58	17.90	20.23	22.40
C	1.100	13.15	15.50	17.87	20.15	22.40
D	1.287	13.29	15.70	18.00	20.38	22.55
E	1.470	13.38	15.60	18.00	20.35	22.55

Table II

Polystyrene standards used from Pressure Chemical Co. and their polydispersity

Molecular weight $\bar{M}_n$ (gm/gm-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



(5) A comparison of the elution peaks measured in SEC experiments run at 1 cm^3/min (----) and 3 cm^3/min (——). Polystyrene ($\bar{M}_N$ = 2.85 x 10^6 and $\bar{M}_N$ = 9.0 x 10^5) in THF run in the DuPont SE-1000A column.

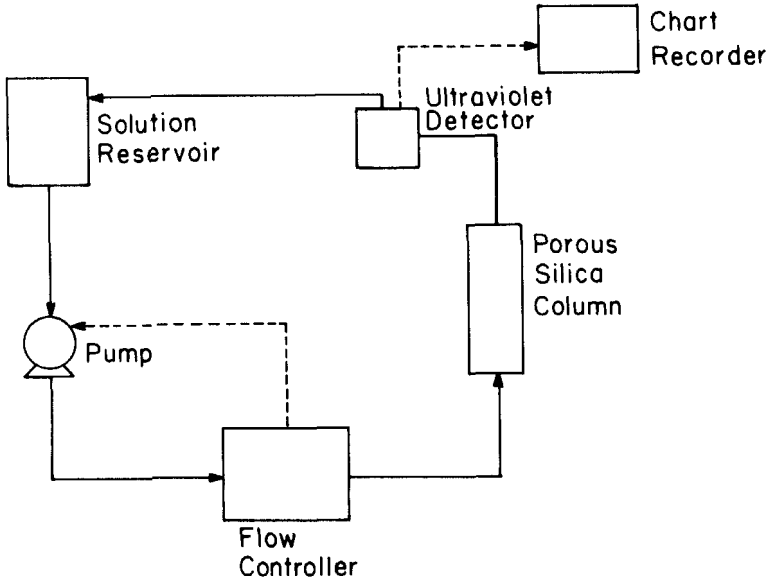
a flow rate of 1 cm^3/min the peak elution volume was 3.57cm^3 and the elution volume of the first moment was 3.79cm^3, while at 3 cm^3/min the peak elution volume was 3.90cm^3 and the elution volume of the first moment was 4.15cm^3. Hence, the elution volumes were found to increase with flow rate. These results and the results of Gudzinowicz and Alden cannot be explained by any of the mechanisms discussed so far, since all of these mechanisms result in elution volumes decreasing with increasing flow rate.

At the same time that these chromatography measurements were made at different flow rates, steady state measure-

ments (independent of time and diffusion rates) were made on the partition coefficient with the same columns and instrumentation, the same polymer/solvent system, and the same range of flow rates.[43] The purpose of these experiments was to determine the origin of flow rate dependent elution volumes in these systems.

These experiments involved pumping polymer solutions continuously through one of the SEC columns and monitoring the effluent concentration (Fig. 6). At a predetermined time the flow rate through the column was changed instantly and the effluent concentration monitored. A typical response of the detector and recorder to a step flow rate change is shown in Fig. 7. The solution used in this example was 0.053 wt % PS ($\bar{M}_N$ = 1.8 x 10^6) in THF. The flow rate was changed first from 3 to 1 cm^3/min and, at a later time increased back to 3 cm^3/min. After the flow rate decrease the effluent concentration was higher, which indicated that the column retention was lower at the lower flow rate. The opposite occurred when the flow rate was again increased. These results indicate the partition coefficient (for partitioning between the interstitial volume in the column and the micropores of the stationary phase) was dependent on the flow rate through the column and increased as the flow rate increased. Similar observations have been made by Chauveteau and Kohler[44] and Willhite and Dominquez in other types of porous media.

The results of these experiments were compiled as the changed column retention, Γ_{ij}, for a given flow rate change from i to j for various molecular weights. These results are summarized in Fig. 8. The apparent dependence of the partition coefficient K_D, on flow rate can be determined from these experimental results by

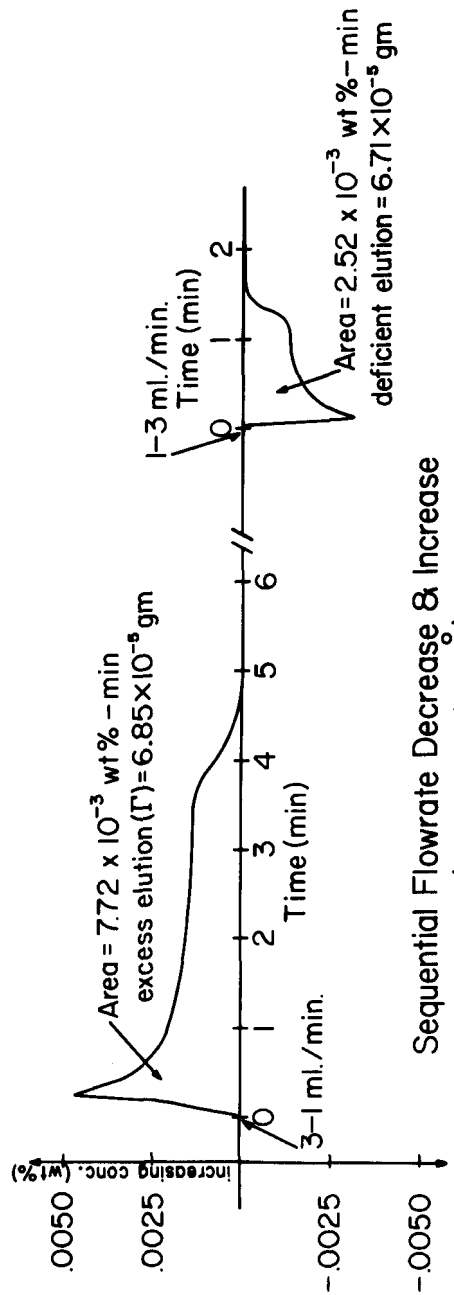


(6) Schmatic of the high pressure liquid chromatograph flow path. Solid lines are flow paths and dashed lines are information paths.

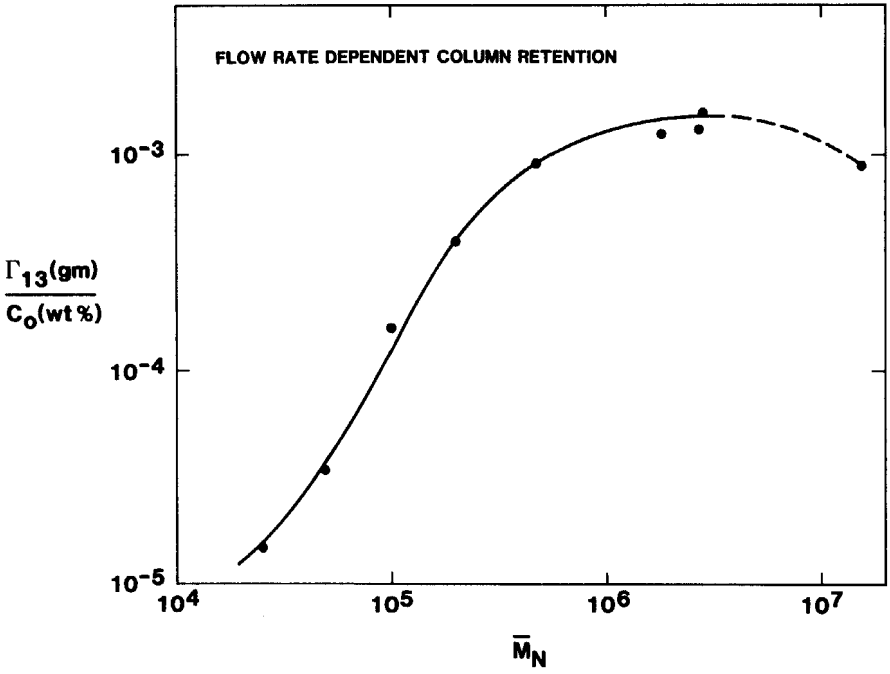
$$K_D(Q_j) = K_D(Q_i) + \frac{\Gamma_{ij}}{C_o V_I} \qquad (3)$$

where C_o is the concentration being fed to the column and V_I is the total volume of micropores. The flow rate dependent partition coefficients predicted from Eq. (3) agree well with those measured in dynamic chromatography experiments,(21,35) (Eq. 1). Table III shows this comparison.

The mechanism causing the apparent flow rate dependence of the equilibrium partition coefficient has not been conclusively determined. One possible mechanism has been proposed which is in good qualitative agreement with all of the experimental observations.(46) The basis of the mechanism is the migration



(7) Typical detector responses to step flow rate changes, (3-1 cm^3/min and 1-3 cm^3/min), for PS ($\bar{M}_N$ = 1.8 x 10^6) in THF in the 1000A column.



(8) Plot of log (Γ_{13}/C_o) versus long (M_N) $\bar{f}$or PS in THF in the 1000A column. The flow rate changes were between 1 and 3 cm^3/min.

of macromolecules which may occur in the nonhomogeneous and curvilinear velocity field of the interstitial volume. The magnitude and the origin of this macromolecular migration has been described for the Rouse model of a macromolecule and can be summarized by the following equation:

$$\underline{v}^{drift} = \frac{1}{6} \langle \underline{R}\,\underline{R} \rangle : \nabla\nabla\underline{v} + \cdots \qquad (4)$$

The time average migration, or drift velicity of the macromolecule's center of mass, relative to the time averaged

Table III. Measured and predicted dependence of elution volume on flow rate in the DuPont silica columns.

Measured Quantities					Predicted Quantities	
$\bar{M}_n$	Elution Volume, ml		K_D	Γ_2/c_o	K_D	Elution Volume
	Q=1	Q=3	Q=1	1-3ml/min	Q=3	Q=3ml/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

solvent velocity, $\underline{v}$, is $\underline{v}$ drift, and $\langle \underline{R}\ \underline{R} \rangle$ is the configurational average of the dyadic product of the macromolecules' end-to-end vector. Recently a similar result has been obtained for the Zimm model by incorporating hydrodynamic interactions between different parts of the macromolecule.(47) In a non-homogeneous and curvilinear flow field, such as in a porous media flow, Eq. (4) predicts that macromolecules would migrate to the concave side of the streamlines or toward the surface of the stationary phase in a chromatographic column.(46) This would tend to build up the macromolecular concentration at the stationary phase surface. Since the migration velocity is flow rate dependent, increasing with flow rate, the surface concentration increases with flow rate also.

If equilibrium partitioning occurs between the surface concentration and the micropore concentration, then the micropore concentration is also flow rate dependent. This is equivalent to the equilibrium partition coefficient having an apparent flow rate dependence. This has been the only mechanism proposed to explain the observation of retentive volumes increasing with flow rate in a chromatographic column. Although this mechanism has not been proven to be a cause of flow rate dependent retention, it is consistent with all of the experimental observations.(46)

8. Conclusions

In this review we have summarized, in a mechanistic way, experimental data which show that elution volumes can depend upon the flow rate through an SEC column. The flow rate dependence of elution volumes can be caused either by anomalous effects or physical effects which result in a change in the

distribution of molecules between the interstitial volume in the column and the micropores of the stationary phase. Nonequilibrium effects (i.e. equilibrium not being maintained at the surface of the stationary phase) have been shown both theoretically[28] and experimentally[22-24] to be of no significance in SEC.

A number of anomalous effects have been documented which result in apparent flow rate dependent elution volumes. These include the following:

1) Errors can be caused by estimating the first moment of the elution curve by the position of the peak. These differ if the elution curve is skewed, and the amount of skewing depends upon the flow rate through the column.
2) High molecular weight species can be degraded in the columns if the deformation rates are high enough. The amount of degradation increases with flow rate, which can result in an apparent increase in elution volumes with flow rate.
3) Instrumental anomalies can result in apparent flow rate dependent elution volumes if the instrumental performance depends upon flow rate. The best example of this involves the use of a siphon to collect the effluent and measure the flow rate. The siphon performance depends upon flow rate due to solvent evaporation in the discharge chamber and to the fact that flow continues into the siphon as it is discharging.

A number of physical effects operate in a chromatograph which can result in a change, with flow rate, in the

distribution of molecules between the interstitial volume in the column and the micropores. These include the following:

1) The possibility exists that the stationary phase of an SEC column which is packed with a nonrigid packing can change with flow rate and pressure drop through the column. This change could involve either the micropore shape or volume decreasing as the pressure drop increases, which would result in elution volumes decreasing with increasing flow rate.

2) Concentration/flow rate effects can result in flow rate dependent elution volumes in two ways. The first way is because the equilibrium partition coefficient depends upon cencentration and the average concentration in the solute band depends upon flow rate. At high flow rates the width of the solute band is greater and hence the average concentration in the band is slower. This effect results in elution volumes decreasing as the flow rate is increased. The second way that flow rate dependent elution volumes can occur is if viscous fingering happens in flow through the column. The amount of viscous fingering is flow rate dependent, and the elution volume, and also the shape of the elution curve, depends upon viscous fingering. This effect is of no significance unless the viscosity of the injected solution differs substantially from the solvent viscosity.

3) Molecular migration phenomena can result in the surface of the stationary phase being exposed to an enhanced concentration of solute as the solute band passes.

The enchanced concentration results in greater partitioning into the micropores and a greater elution volume. Since the migration phenomena increases with flow rate, this effect results in elution volumes increasing as the flow rate increases. This mechanism has not been totally justified, but it results in predictions that are in qualitative agreement with data obtained or flow rate dependent elution volumes in column with rigid packings.

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