# Isolation of Peak Broadening Factors in Exclusion (GEL) Chromatography

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ABSTRACT: The problems and importance of isolating fundamental peak dispersion terms in exclusion chromatography (EC) are discussed, and the approaches suggested by earlier work in gas chromatography are noted. The theory of peak dispersion (plate height) in EC is formulated from general chromatographic theory. Strategies for isolating adsorptive terms and stationary phase diffusion terms are then established. The obstruction factor representing the hindrance to diffusion in the pore space is discussed, and theoretical reasons are given for expecting a reduction in this factor, due to changes in the constriction effect, under conditions practical to EC. Measurements from one of our earlier papers, combined with new experimental data, are applied to the theoretical framework. It is shown, first of all, that retention is independent of the diffusion coefficient, thus directly contradicting the restricted diffusion model of retention and lending support to the equilibrium model. Evidence is then shown for a small adsorptive term in the plate height of our EC system using two liquid solvents and a larger term with a dense gas solvent. From this, a desorption rate parameter is estimated. Estimates are then made of the nonequilibrium term for stationary phase diffusion for several solutes. An obstruction factor for diffusion of 0.17 is obtained, and the validity of this result is discussed in the light of theory and independent experiments.

The broadening of monodisperse solute peaks in exclusion chromatography (EC) is a matter of great importance in the effort to obtain high resolution separations and molecular weight distribution curves. Accordingly, a large number of theoretical[1-6] and experimental[7-11] studies of the phenomenon have been undertaken. The advances in our basic understanding, however, have been limited because it has not been possible to make a term by term comparison of theory and experiment. Theory provides a group of contributing peak dispersion terms, where each term is numerically uncertain because of unknown factors which describe mass transport in the complex flow and pore space of an EC column. Experiment yields the sum of such terms. Unfortunately, it is difficult to isolate the various factors that contribute to experimental band broadening to enable a term by term comparison with theory. Accordingly, only limited progress has been made in isolating and analyzing the basic contributions to peak dispersion in EC. Without such a fundamental comparison, the potential role of theoretical guidelines in developing improved column systems is seriously impaired. Put another way, if the principal origins of band broadening are unknown, no direct attack can be made to reduce them.

Workers in gas chromatography (GC) faced a similar problem in the early 1960's. A number of approaches were developed to isolate various contributions to GC band broadening.[12-18] As a consequence, a much more detailed picture of molecular transport processes in GC packings and liquid phases emerged.

Many of the approaches developed for GC studies are applicable to all forms of chromatography, including EC. A typical technique, and one of the most successful, is based on experiments in which several (or the same solvent under several conditions) carrier solvents are used alternately in the same column.[16,18] The solute–solvent diffusivity, of course, is different for each solvent. If diffusivity values are known, it is possible to isolate all of the terms that depend on, and are scaled to, solvent diffusivity. If there are residual terms that hinge on adsorption, interfacial diffusion, etc., these then become discernible. If column processes are proceeding without such contributions, this will be indicated by the superposition of peak dispersion curves when plotted in the appropriate reduced coordinates.[19]

The above subtraction process was first used in GC to obtain nonequilibrium terms for the stationary phase. Solute-gas diffusion coefficients were varied by either gas substitution[16] or by its equivalent, outlet pressure variations.[16,18] In the latter, of course, appropriate compressibility corrections are needed.[18] In another study, the comparison of dispersion gas and liquid solvents in the same column yielded evidence concerning dispersion by turbulence and coupling at high fluid velocities.[20]

It should be noted that, along with peak dispersion data, solvent substitution in EC will yield results which have a bearing on the equilibrium vs. restricted diffusion theories of solute migration. The equilibrium concept is by far the most plausible in the light of both theoretical and experimental studies.[21-24] By making substitutions of solvent in which nothing significant varies except the diffusion coefficient, however, one can obtain further evidence on this fundamental question.

Additional information on basic EC processes can be obtained by comparing two different columns, one filled with porous support material and one with impermeable support material. Work of this type has been done by Kelley and Billmeyer.[5] The difference in the band spreading observed will provide an estimation of the peak broadening arising from slow diffusion and/or kinetics within the particles. This can be compared with values from well established chromatographic theories.[19] The drawback of the method is that different columns (especially those filled with different materials) never pack identically. We shall obviate this problem here by using nonpermeating species in the same column. This is analogous to the GC technique of using *inert* peaks or *air* peaks.

Recently, we have reported the successful operation of an EC column using a solvent consisting of a highly compressed gas.[25] Ordinary liquid solvents were used in the same column to prove the exclusion mechanism. In the course of that work, multisolvent data of the type mentioned above were acquired. Data on nonpermeating species were also obtained. The implications of those data, regarding basic peak dispersion mechanisms, are studied here. In addition, new data have been acquired on a column of nonporous particles. These data compliment the multisolvent experiments in analyzing contributions to peak dispersion.

## Theory

Although the application of general chromatographic theory to band broadening in EC was first made about a decade ago,[1] very few controlled experimental results were then available for critical evaluation. The key factors in that theory appear

below, but the approach has been expanded considerably to suit our present purposes.

Peak dispersion in this paper is expressed in terms of *plate height*, a convention in the chromatographic literature. Plate height, however, is not connected to any model of discrete plates, as in a distillation column. It is, instead, a coefficient representing the rate of generation of variance, $\sigma^2$, in zone width and respect to the distance $Z$ traversed by the zone in the column: $H = d\sigma^2/dZ$. As such, it is related to the dispersion coefficient (effective diffusion coefficient), $\mathcal{D}$, by[19]

$$H = \frac{2\mathcal{D}}{\mathcal{V}} = \frac{2\mathcal{D}}{Rv} \tag{1}$$

where the mean solute velocity $\mathcal{V}$ is also expressible as $Rv$ where $v$ is the mean mobile phase (interparticle) velocity and $R$ the retention ratio.

A large number of mechanisms have been identified as contributing to plate height $H$ in chromatography.[19] Most of these are associated with processes by which solute is transferred between microscopic regions differing in the rate of displacement by flow. The contribution to $H$ is generally largest for those processes that transfer solute most slowly, a fact that can be deduced by simple random-walk arguments.[19] The limited rate of such transfer processes allows a partial depletion of solute in sole regions. This creates a small departure from local equilibrium and leads to the descriptive term "nonequilibrium" in describing such processes. The direct relationship between the degree of nonequilibrium and that of band broadening has been described.[19]

Many plate height mechanisms operative in partition and adsorption chromatography are absent in EC, at least in the *ideal* case in which surface interactions are absent. Thus, interfacial transfer, adsorption–desorption processes, and diffusion in a second, chemically distinct phase are normally absent. The following processes remain which can be identified with ideal EC:

**1. Longitudinal Diffusion.** The axial component of solute diffusion contributes directly to $H$.

**2. Eddy or Multipath Diffusion.** Randomly packed particles force the repeated restructuring of the microscopic stream pattern, thus effectively causing the exchange of solute between various flow regimes at frequent intervals.

**3. Nonequilibrium Diffusion.** Diffusion in the solvent, both in and out of the pore space, leads to the transport between distinct flow regimes. (This process gives rise to several terms, each identified with a particular region of column space.)

Cases of nonideal EC may exhibit additional plate height terms. Two such terms related to adsorption are mentioned below; others undoubtedly exist but are, in all likelihood, not often important.

**4. Adsorption–Desorption Nonequilibrium.** The speed of any adsorption–desorption processes will be a contributing factor in determining the overall rate of transport between mobile and stationary phases.

**5. Surface Diffusion.** The diffusion of adsorbed species along the surface will augment the transport of solute by bulk diffusion in and out of the pore space.

It remains now to account for these processes by means of a realistic plate height equation. For this, we turn to well established chromatographic theory.

A rather general expression for plate height in a chromatographic column is[19]

$$H = C^s v + \frac{B}{v} + \sum_i \frac{1}{(1/A_i) + (1/C_i^m v)} \tag{2}$$

where $B$ is the longitudinal diffusion coefficient (deriving from item 1 in the classification of processes above) and the $A_i$'s are eddy diffusion terms related to the erratic transport of solute in a packed bed (cf. category 2 above). (All terms are defined more precisely elsewhere.[19]) Superscripts s and m refer to stationary and mobile phases, respectively. The summation satisfies the need to account for the contribution of different classes of flow inequalities in the granular medium.

The $C$ coefficients are the nonequilibrium terms (3 through 5 in the classification above). Those related to diffusion between velocity regimes in the mobile phase can effectively be "short circuited" by eddy diffusion; they are therefore coupled to the corresponding $A_i$ terms in the summation in the same way that terms for parallel resistors combine. The uncoupled term $C^s$ is related to processes in the stationary (pore) space. These processes are insulated from significant convective displacement by the large flow resistance of the porous network. In cases of extremely high flow velocities, however, the component $C^s$ terms would also need to be written in coupled form.

The coupling expression (the summation term of eq 2) is simply an approximation to a very complex interaction between diffusion and convection.[19] The process has been formulated rigorously, but the boundary conditions for real granular beds are so complex that meaningful solutions have not yet been obtained.[26,27]

Ideally, nonequilibrium in the stationary phase (intraparticle or pore space) is controlled by diffusion that can be considered homogeneous on the scale of the particles and is unhindered by adsorption effects. The effective diffusion coefficient is $\gamma_s D$, where $D$ is the bulk solute–solvent diffusion coefficient and $\gamma_s$ is an *obstructive factor* for the pore network of the stationary phase. Such diffusion in a bed of identical particles leads to the equation

$$C^s_{diff} = qR(1 - R)d_p^2/\gamma_s D \tag{3}$$

where $d_p$ is a characteristic particle dimension, $q$ is the *configuration factor* for that type of particle, and $R$ is the retention ratio. For spheres of diameter $d$, $q = 1/30$; other values have been determined theoretically for different geometries.[19]

If the packing consists of particles of unequal size or shape, the general *combination law* specifies that one must apply the volume-weighted average value, $qd_p^2/\gamma_s$, in evaluating eq 3.[19,28] Therefore, for a collection of spheres of volumetric rms diameter $\bar{d}_p$, in which $\gamma_s$ and $q$ are constant, we have

$$C^s_{diff} = \frac{R(1 - R)\bar{d}_p^2}{30\gamma_s D} \tag{4}$$

This equation, of course, can be applied to collections of nonspherical particles if $\bar{d}_p$ is properly associated with the correct mean particle dimension. For irregular particles reasonable values of $\bar{d}_p$ may be difficult to estimate, but the value chosen should lean toward the effective width, rather than length, because equilibration will occur mainly along the shortest possible path.

If the EC process is made nonideal by adsorption, and if the adsorption–desorption kinetics are sluggish enough to create an additional peak dispersion effect, the extra contribution to $C^s$ can be calculated exactly for complicated multistep kinetics by employing eq 4.2–31 from ref 19. However, eq 4.6-37 is more practical in form, and adequately descriptive. This equation gives

$$C^s_{ads} = 2R(1 - R)\phi/k_d \tag{5}$$

where $k_d$ is the effective rate constant for desorption and $\phi$ is the fraction of the solute in the pore network that exists in the "adsorbed" state.

The foregoing material establishes in fairly general terms the nature of band broadening in EC. It fails, however, to show how the magnitude of various plate height terms can be isolated experimentally. For example, the relative contribution of $C^s_{diff}$ and $C^s_{ads}$ is masked by their identical dependences

on $R$ and their lack of dependence on $v$. The unique dependence of $C^s_{diff}$ on particle diameter $\bar{d}_p$ is difficult to utilize because different columns, particularly with different size particles, are virtually impossible to pack into a geometrically similar microscopic flow structure. The unequal dependence of the two terms on $D$ does not provide an obvious route either because most other plate height terms (notably $B$ and $C_i^m$) also change with changes in $D$. The special form in which these various terms depend on $D$, however, makes it possible to separate $C^s_{ads}$ from other plate height terms and at the same time to check the ideality of the EC process. Techniques based on first the $R$ dependence and second the measurement of plate height in nonpermeable packings permit the isolation of $C^s_{diff}$ from other plate height terms, as will be illustrated shortly. The $B$ term can be isolated by virtue of its unique velocity dependence, but this term is not of sufficient importance in EC to merit the tedious, low-velocity work necessary for its determination.

We now introduce reduced coordinates: the reduced plate height is $h = H/d_p$, and the reduced velocity is $\nu = d_p v/D$.[19,29] By using the arguments developed in our previous paper,[1] it can be shown that the value of $h$ in ideal EC, given a fixed packing, solute, and pore geometry, is a function only of $\nu$. Thus if plate height curves are acquired for different solvents used with the same solute in the same columns, the $h$ vs. $\nu$ curves should superimpose, providing the column processes are unperturbed by surface and other extraneous effects.

If, however, $C^s_{ads}$ is finite and independent of solvent, the reduced plate height equation acquires the form

$$h = h_{id}(\nu) + C^s_{ads}\nu(D/d_p^2) \tag{6}$$

where $h_{id}(\nu)$ represents the $h$ curve for ideal EC. We now follow the general outlines of a procedure developed in 1964 to isolate the plate height term of gas chromatography.[18] From the separate $h$ vs. $\nu$ curves for two solvents, we identify the difference, $\Delta h$, in reduced plate height values at a fixed value of $\nu$. This can be identified with the following increment derived from eq 6

$$\Delta h = (C^s_{ads}\nu/d_p^2)\Delta D \tag{7}$$

where $\Delta D$ is the increment in diffusion coefficient, $D$. The $h_{id}(\nu)$ term has dropped out, of course, because $\nu$ is stipulated to be identical for the two points. Equation 7 leads to the following equation for $C^s_{ads}$

$$C^s_{ads} = \frac{d_p^2}{\nu}\frac{\Delta h}{\Delta D} \tag{8}$$

All terms in this equation can be obtained by direct measurement or by independent means, thus providing a route to identifying the magnitude of $C^s_{ads}$ in nonideal EC.

It should be noted that the $C^s_{ads}$ term so derived is extremely general, not at all limited to the mechanism described by eq 5. It represents any nonequilibrium term unaffected by solvent arising in the stationary regions of the column. We should note some caution in applying this to adsorption–desorption kinetics, however, as the rate of desorption may depend on the nature of the solvent. In the most extreme case of this dependence, the $C^s_{ads}$ term may be large for one solvent and virtually absent for others. In this case eq 8 must be replaced by

$$C^s_{ads} = \frac{d_p^2}{\nu}\frac{\Delta h}{D_2} \tag{9}$$

where $D_2$ is the diffusion coefficient of solute in the solvent in which $C^s_{ads}$ is important.

A different strategy must be used to isolate the nonequilibrium term for pore network diffusion, $C^s_{diff}$, described in eq 4. First, the ideal reduced plate height of eq 6, $h_{id}$, should be isolated from $C^s_{ads}$ by the procedure just outlined. We now write the ideal component in terms of its mobile phase and stationary components

$$h_{id} = h_{id}^m + h_{id}^s \tag{10}$$

The $h_{id}^m$ curve can be obtained (a) by using a solute that cannot permeate into the pore network or (b) by making measurements on a comparable column packed with nonpermeable particles. Method (b) was used by Kelley and Billmeyer to separate plate height terms.[5]

The term $h_{id}^s$ is now obtained as $h_{id} - h_{id}^m$. Inasmuch as $h_{id}^s = (H_{id}^s/d_p) = C^s_{diff}v/d_p = C^s_{diff}\nu D/d_p^2$, the desired parameter $C^s_{diff}$ can be identified

$$C^s_{diff} = h_{id}^s d_p^2/\nu D \tag{11}$$

where $D$ is the diffusion coefficient of the permeating species of interest.

The use of solutes that do not penetrate the stationary phase, as outlined above, has also been of considerable utility in the analysis of plate height in gas chromatography. The nonsorbing peaks have been termed air peaks or inert peaks, as noted earlier.

We turn briefly now to the matter of the obstruction factor $\gamma_s$, which appears in eq 3. This factor represents the hindrance to solute diffusion in the pore network. This kind of hindrance is important in many fields involving transport processes in porous media. We wish to raise the possibility that macromolecules in tight fitting pores, typical of EC, are subject to a larger $\gamma_s$ than are small molecules in equivalent networks.

It has been shown that obstruction factors are composed of two terms of roughly equal magnitude: a *tortuosity* factor representing the increased pathlength for transport, and a *constriction* term representing "bottlenecks" to effective transport.[30–32] An equation describing these effects is[19]

$$\gamma_s = [\tau^2\overline{A}(\overline{1/A})]^{-1} \tag{12}$$

where $\tau$ is the tortuosity and $A$ is the cross-sectional area available for transport. The product of averages, $\overline{A}(\overline{1/A})$, accounts for the constriction effect.

The role of constriction is not generally well understood, and its contribution is often attributed, erroneously, to tortuosity. The distinction is especially important in EC, because the constriction effect may be multiplied as a result of the comparable size of molecules and pores.

The constriction effect arises from the fact that constrictions in the diffusion path will reduce the solute quantity available for transport and will thus diminish solute flux. Under conditions applicable to EC (molecular dimensions comparable to pore dimensions), the relative quantity of solute in the bottlenecks is expected to be reduced even more by the unfavorable entropy which serves to discourage macromolecules from entering constricted regions of the pore network. (The entropy effect is more generally responsible for the basic EC effect of selective partitioning into the pore space.[21–24]) Effectively, the solute available for transport through pore bottlenecks is reduced, first, by the reduced cross-sectional area, and second, by the reduced concentration. The latter varies in proportion to the local distribution coefficient $K$, representing the solute concentration in pore segments of the specified degree of constriction relative to that in bulk solvent. Under these circumstances, it is possible to show that a more suitable equation for $\gamma_s$ is

$$\gamma_s = [\tau^2\overline{AK}(\overline{1/AK})]^{-1} \tag{13}$$

This will yield a smaller value for $\gamma_s$ than eq 12 because $A$ and $K$ are correlated, as mentioned above. For small molecules in the pore space between randomly packed glass beads, the obstructive factor is $0.60 \pm 0.02$.[32] This value is probably

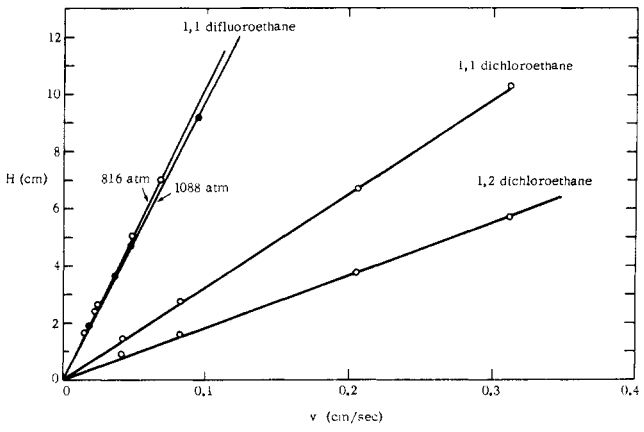


**Figure 1.** Plate height vs. velocity for 4000 mol wt polystyrene in an open tube of circular cross section. Lines are least-squares plots forced through the origin. The slope provides the diffusion coefficient (reported in Table I) for the corresponding solvent. The 1,1-difluoroethane lines apply to the dense gaseous state at 118 °C and at the designated pressure.

typical for porous media of a porosity comparable to that for glass beads. It is not unreasonable in view of the above, however, to expect $\gamma_s$ values to be considerably smaller than 0.6 for EC. We will return to this matter in the discussion of experimental data.

## Experimental Section

The equipment and materials used to acquire data using liquid solvents and using dense gas solvents has been described in detail elsewhere.[25] The stainless steel EC columns, fitted to be interchangeable between liquid solvent systems and dense gas systems, were 103 cm in length by 0.203 cm in inside diameter. A shorter column (13.5 cm) was used to correct for end effects, following a procedure developed for that purpose.[33] The EC support was 40A porous silica glass from Electronucleonics, sieved to 44–74 $\mu$ particle diameter. The mean diameter was assumed to be 59 $\mu$m. Solvents were 1,1-dichloroethane, 1,2-dichloroethane, and tetrahydrofuran at 25 °C and dense gaseous 1,1-difluoroethane at 118 °C.

A glass bead column was also used in the present study. This was packed with 200–325 mesh (44–74 $\mu$m) glass beads from English Glass Ltd. The beads were treated with hexamethyldisilazane to suppress adsorption. The method of preparing the column and correcting for end effects was identical to that used for the EC column.[25] Column dimensions were also the same.

Diffusion coefficients were measured on a select group of solute–solvent systems so that the data analysis outlined in the theory section could be made. The basic procedure developed by Giddings and Seager for measuring gaseous diffusion coefficients was used,[33] with some modification for liquids as outlined by Grushka and Kikta.[34] The length of the open stainless steel tube employed for this purpose was 525.4 cm, and the inside diameter was 0.406 cm. These tubes, like the EC columns, were made interchangeable between liquids and dense gases by high-pressure fittings. Several samples were used at each linear velocity. The plate height–velocity data were fitted by a straight line passing through the origin using a linear least-squares regression. The axial diffusion term was ignored and the slope of the line was therefore identified with the term $R_0^2/24D$, where $R_0$ is the tube radius.

## Results and Discussion

**Diffusion Coefficients.** Since diffusion coefficients are essential for a detailed plate height analysis, but are not readily available in the literature for the solute–solvent systems employed here, we have measured critical $D$ values by the method outlined in the experimental section. Figure 1 shows the plate height–velocity plots from which the $D$'s have been obtained. The data acquired at different velocities are seen to follow closely the expected proportionality between $H$ and $v$; this lends confidence in the validity of the results. The four $D$ values acquired by this method are shown in Table I.

Diffusion coefficients for other polystyrenes have been calculated from the 4000 mol wt material by assuming that $D \propto (\text{mol wt})^{-0.5}$. The relatively small mol wt range employed justifies this approximation. Toluene diffusivity in the different solvents was obtained by multiplying the $D$ value reported for chloroform as a solvent, $2.01 \times 10^{-5}$ cm$^2$/s,[34] by the ratio of solvent viscosity to chloroform viscosity. The result for toluene in 1,1-dichloroethane at 25 °C is $D = 2.35 \times 10^{-5}$ cm$^2$/s.

**Equilibrium vs. Restricted Diffusion Models of Retention.** Retention volumes in EC are usually independent of flow velocity. This is considered to be strong evidence in favor of the predominant role of equilibrium in determining retention.[21-24] The results in Table II show that the retention volumes obtained in this study are independent of solvent as well as velocity. In that each solute's diffusion coefficient, $D$, varies with solvent, this result is tantamount to showing that

**Table I**
**Diffusion Coefficients for 4000 mol wt Polystyrene Solute Measured by the Chromatographic Method**

| Solvent | $T$, °C | $p$, atm | State | $D$, cm$^2$/s |
|---|---|---|---|---|
| 1,1-Dichloroethane | 25 | 0.84 | Liquid | $5.80 \times 10^{-6}$ |
| 1,2-Dichloroethane | 25 | 0.84 | Liquid | $3.44 \times 10^{-6}$ |
| 1,1-Difluoroethane | 118 | 1088 | Dense gas | $10.8 \times 10^{-6}$ |
| 1,1-Difluoroethane | 118 | 0.816 | Dense gas | $11.2 \times 10^{-6}$ |

**Table II**
**Retention Volume in ml for Polystyrene and Toluene Solutes in Three Different Solvents at the Flow Rate of 16.2 ml/h[a]**

| Polystyrene mol wt | Retention vol, ml | | | | |
|---|---|---|---|---|---|
| | THF | $CH_3CHCl_2$ | $CH_2ClCH_2Cl$ | $CH_3CHF_2$ | R |
| 20 400 | 1.64 | 1.65 | | 1.66 | 1.00 |
| 10 400 | 1.72 | 1.72 | | 1.72 | 0.96 |
| 4 000 | 1.85 | 1.84 | 1.84 | 1.85 | 0.90 |
| 2 100 | 1.91 | 1.91 | | 1.92 | 0.86 |
| 600 | 2.05 | 2.04 | | 2.05 | 0.81 |
| Toluene | 2.25 | 2.25 | | 2.24 | 0.73 |

[a] Tetrahydrofuran, 1,1- and 1,2-dichloroethane are liquids at 25 °C; 1,1-difluoroethane is a gas at 118 °C and 1088 atm of pressure. The retention ratio, $R$, is calculated from the 1,1-dichloroethane results. The volume of the column without support is 3.29 ml.

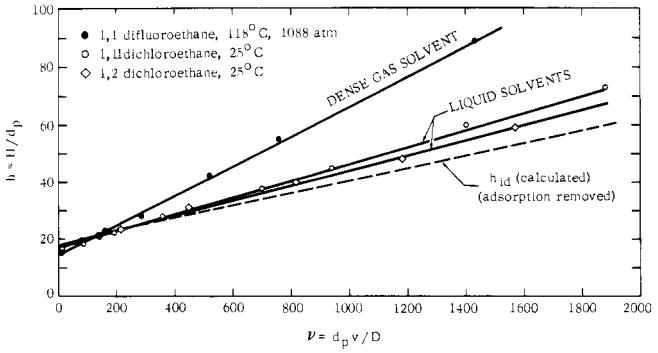


**Figure 2.** Reduced plate height vs. reduced velocity for 4000 mol wt polystyrene in the EC column using the solvents indicated. Plots are obtained by least squares. The ideal (adsorption free) line was calculated from the two liquid lines using $h_{id} = h - D\Delta h/\Delta D$.

retention volumes are independent of diffusion coefficient.

The distribution of $D$'s for any given solute can be estimated from the solvent viscosities: 0.453, 0.469, and 0.265 cP for THF, $CH_3CHCl_2$, and $CH_3CHF_2$, respectively.[25] In the case of 4000 mol wt polystyrene, the $D$'s have been measured directly for several solvents, as reported in Table I.

The observation of constant retention volumes for each solute independent of solute–solvent diffusion coefficients provides the most direct evidence that diffusion has little bearing on retention. This evidence complements the flow-velocity independence noted above. Neither result is consistent with the concept that incomplete diffusion into the pore network is the controlling factor in EC retention.

**Reduced Plate Height Plots. Isolation of Adsorption Terms.** As noted in the Theory section, ideal EC (without adsorption) will yield plate height plots that superimpose when plotted in the reduced coordinates, $h = H/d_p$ and $\nu = d_p v/D$. Figure 2 may therefore be considered as a test of EC ideality for 4000 mol wt polystyrene solute. The near superposition of plots for the two liquid solvents provides strong evidence for a condition close to ideality in those particular solvents. However, a finite divergence is apparent, suggesting some adsorptive effect. The marked divergence from the liquid curves of the plot for the dense gas solvent, 1,1-difluoroethane, suggests that nonideal influences have a significant role in this solute–solvent support combination.

The most obvious explanation of nonideality is reversible adsorption of some form. It is clear that adsorption would tend to alter retention volumes as well as peak dispersion. However, retention volumes in the dense gas and liquid solvents are identical, within measurable limits. It appears, therefore, that plate height, measured under our experimental conditions, is far more sensitive to adsorption than is retention. Further experimental support for this observation is found in our earlier paper, where relatively low pressure dense gas EC was found to produce extremely broad peaks without significantly affecting retention volumes.[25] We show below that this phenomenon is consistent with theory.

For both retention and plate height, adsorption is expected to add a term proportional to the fraction $\phi$ of stationary phase solute that is adsorbed. In the case of retention, in which retention volume $V_r$ is ideally $V_m + KV_s$, the added term is $KV_s\phi/(1 - \phi)$. In the case of plate height, the added term would be one approximated by eq 5. Examination of these two terms shows that there is nothing about them requiring that they have an equal fractional influence on retention and plate height, respectively. A small $\phi$ combined with a small desorption rate constant, $k_d$, can produce a substantial contribution to plate height, but only a negligible change in retention volume. The experimental evidence lends support to this condition of the adsorbed state in our columns.

The value of the adsorptive nonequilibrium term, $C^s_{ads}$, can be established by the procedures outlined in the Theory section, providing the data are accurate enough to support calculations based on the differences between the two curves. This is clearly the case for the curve for the dense gas solvent with respect to either of the other experimental curves in Figure 2. The two liquid curves, however, are close enough that experimental plate height errors and small errors in measured diffusion coefficients could significantly alter conclusions based on the increment between curves. We proceed with the calculation, nonetheless, because it is illustrative of the type of detailed analysis that can be applied to EC.

The ratio of the increment $\Delta h$ in the two liquid curves to the reduced velocity $\nu$ in Figure 2 varies slightly with $\nu$, in contradiction to theory. We arbitrarily select values at $\nu$ = 1400 for our calculations. At this point the value of $\Delta h/\nu$ obtained from the least-squares lines is 0.002 87. The application of eq 8, with the help of $D$ and $d_p$ values found elsewhere in this work, yields $C^s_{ads}$ = 0.042 s. This value is assumed by the theory to be equal for both solvents.

The adsorptive nonequilibrium term obtained above can be subtracted from the overall $h$ to yield the ideal (adsorption free) curve, $h_{id}$. Our procedure for this is roughly equivalent to that suggested by eq 6, but it accounts at each point for the slight variation in $\Delta h/\nu$ values noted above by its use of the equation $h_{id} = h - D\Delta h/\Delta D$, where $D$ and $h$ apply to the same solvent. The line so generated is the bottom one in Figure 2.

It is possible to translate the $C^s_{ads}$ term just obtained into rate constant information for the adsorption–desorption process by using eq 5. This analysis, however, will be reserved for the dense gas solvent, because the data in that case are more reliable due to the larger increment between curves.

By measuring the increments between the gas and the ideal ($h_{id}$) curves of Figure 1, and then substituting the values obtained into eq 9, the adsorption contribution in the dense gas solvent can be calculated. This procedure, applied at $\nu$ = 1400, yields $C^s_{ads}$ = 0.084 s, a value just twice as large as that estimated for the liquid solvents.

Kinetic parameters can be approached by substituting this value and $R$ = 0.90 (from Table II) into eq 5. The result is $k_d/\phi$ = 2.1 $s^{-1}$. Unfortunately, there is no independent value of $\phi$, thus making it impossible to identify the effective desorption rate constant precisely. Ideally, $\phi$ could be obtained by measuring the adsorption-induced shift in retention volume, but we have noted already that $\phi$ is apparently too small to be measured in this manner. At best, therefore, we can only identify an approximate range for the $k_d$ value.

From the lack of a detectable retention effect, we can conclude that $\phi$ is small, probably $\phi < 0.1$. Thus $k_d$ can be inferred to lie in the range $k_d < 0.2\ s^{-1}$. The mean desorption time for adsorbed polymer, $1/k_d$, is therefore presumably greater than 5 s. An upper bound can be estimated from the procedure below.

The peak width as observed in our laboratory was typically about 100 s. If the mean desorption time exceeds peak width in time units, peak tailing is expected.[19,35] In that no significant peak tailing was observed, it is suggested that an upper bound to the mean desorption time is 100 s. Thus, very approximately, we have $100 > 1/k_d > 5$ s.

In order to establish a more accurate value of $k_d$, high precision experiments would be needed to determine $\phi$ from retention or by other experimental means. The principal value of the analysis above lies in the demonstration that chromatographic evidence can be assembled in a practical way to yield a good deal of information on underlying mechanisms and can establish values or ranges of values for fundamental parameters.

**Isolation of the Stationary Phase Diffusion Term.** We

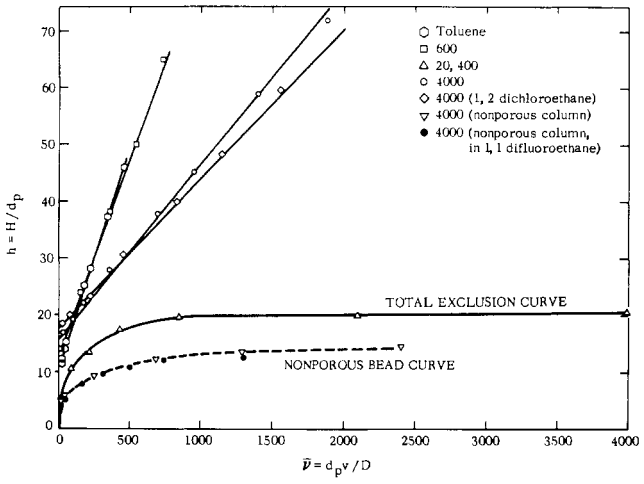


**Figure 3.** Reduced plate height vs. reduced velocity for toluene and for polystyrene polymers of the molecular weights indicated. All curves were obtained from the EC column, except the bottom one, which derives from the porous glass bead column. The solvent is 1,1-dichloroethane in all cases except the ones noted, in which 1,2-dichloroethane and 1,1-difluoroethane are used.

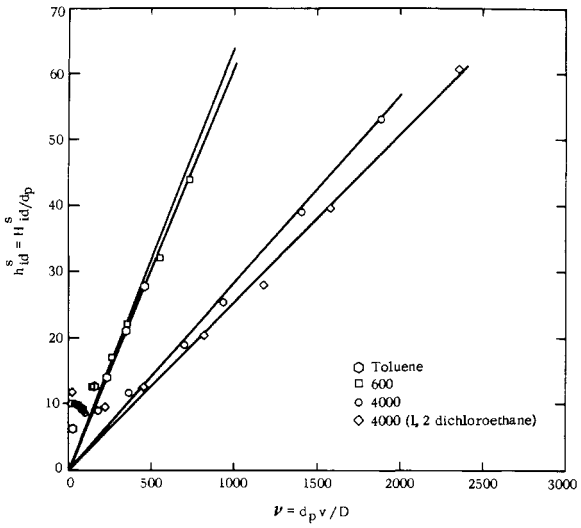


**Figure 4.** Reduced plate heights for the stationary phase obtained by subtracting the *total exclusion curve* from the data for permeation solutes shown in Figure 3. Lines are fitted by least squares; the one farthest left is for toluene.

now follow the procedure outlined in the Theory section to isolate the $C^s_{diff}$ term. Since 20 400 mol wt polystyrene is totally excluded from the pores, the reduced plate-height curve for this solute represents the mobile phase contribution $h_{id}^m$. An independent $h_{id}^m$ curve was obtained by measuring plate heights from the nonporous glass bead columns. Both of these curves are shown in the lower part of Figure 3. The lack of perfect agreement between them is not surprising in that they derive from two different columns and two different supports and in that peak dispersion is generally very sensitive to the structure of the column packing. In this light, the similarity of the two curves tends to reinforce both as a valid measure of mobile phase effects. Their general agreement also confirms the form of the mobile phase curve and suggests the magnitude of the difference for individual columns. The validity of the nonporous bead curve is further confirmed by its virtually identical form in two solvents: liquid 1,1-dichloroethane and dense gaseous 1,1 difluoroethane.

Because the two mobile phase curves do exhibit a measurable difference, it is necessary to choose one of them for the proposed calculations. The curve for the totally excluded solute on the EC column (the upper curve) is undoubtedly the more valid of the two for interpreting the other data obtained on the same EC column. Yau, Malone, and Suchan have called attention to such total exclusion curves and their role in representing only mobile phase processes.[36]

Also shown in Figure 3 are the reduced plate height plots for several permeating species: toluene and polystyrene of both 600 and 4000 mol wt. One set of 4000 mol wt data derives from 1,2- instead of 1,1-dichloroethane. While these two sets of data nearly superimpose, the $C^s_{diff}$ term for the two solvents will be substantially different due to the unequal values of diffusivity that must be substituted into eq 11 to obtain $C^s_{diff}$.

Equation 11 shows that the nonequilibrium term for diffusion, $C^s_{diff}$, is proportional, $h_{id}^s/\nu = (h_{id} - h_{id}^m)/\nu$, where $h_{id}$, by our present assumptions, is represented by one of the upper four solute curves of Figure 3, and $h_{id}^m$ can be taken as one of the two lower curves. In Figure 4 we show the results obtained by subtracting the *total exclusion curve* of Figure 3 from the data points above it, representing the permeating solutes. The points so obtained are fitted with straight lines through the origin; these lines appear in the figure. Clearly the data trend upward near the origin and in so doing depart from theoretical expectations. This is probably due to some slight inaccuracy in the diffusion coefficients or in the *total exclusion curve*. The departure from theory does not appear to be very serious overall, as most of the data demonstrate that there is a reasonable proportionality between $h_{id}^s$ and $\nu$.

The slopes in the lines of Figure 4, $h_{id}^s/\nu$, are used in eq 11 to generate $C^s_{diff}$ values. Table III shows the quantities obtained. The last column shows the $C^s_{diff}$'s corrected for adsorption in the two cases where an adsorption term $C^s_{ads}$ was determined. In these cases, the value, $C^s_{ads} = 0.04$, was subtracted from the $C^s_{diff}$'s in the third column. Presumably, adsorption plays a smaller relative role for the smaller solute species, toluene and 600 mol wt polystyrene, but we have no data to confirm this.

The theoretical expression for the $C^s_{diff}$ terms is given by eq 4. The principal uncertainty in this expression is the obstruction factor $\gamma_s$. A plot of $C^s_{diff}$ vs. $R(1 - R)/D$ should yield a line with a slope of $d_p^2/30\gamma_s$. From this, a $\gamma_s$ term can be extracted. Figure 5 shows such a plot obtained by least squares; the resulting $\gamma_s$ is 0.17. This is smaller than the normal value, 0.6, as suggested in the Theory section.

The fact that there is considerable scatter about the line in Figure 5 suggests either that $\gamma_s$ is not constant, that there are still some unaccounted for adsorptive effects, or that some error in the data has been magnified by the conversion and subtraction steps applied to it. The location of the points for toluene and 600 mol wt polystyrene above the least-squares line implies that $\gamma_s$ might be lower for these components than for 4000 mol wt polystyrene. The inverse of this is expected on theoretical grounds, as noted earlier. Unaccounted for adsorption with these two solutes would explain the anomaly, but significant adsorption is unlikely on the basis of more fundamental considerations.

Colton, Satterfield, and Lai have reported $\gamma_s$ factors based on the measurement of the rate of uptake of solute into leached borosilicate glass cubes.[37] Their results, too, were somewhat erratic but covered many solute–solvent–pore size combinations. They found no systematic variation of $\gamma_s$ with polystyrene molecular weight. The mean value of $\gamma_s$ with polystyrenes ranged from 0.22 for 25 Å radius pores to 0.43 for 185 Å pores. For proteins, by contrast, a decisive decrease in $\gamma_s$ with molecular size was observed.

We note that $\gamma_s$ is expected to vary with porosity on both experimental and theoretical grounds.[30] Our retention and

**Table III**
**Values of the Nonequilibrium Term for Diffusion in the Stationary Phase, $C^s_{diff}$, as Calculated using eq 11 and the Results of Figure 4[a]**

| Solvent | Solute | $C^s_{diff}$, s | $C^s_{diff}$ (corr), s |
|---|---|---|---|
| 1,2-Dichloroethane | 4000 | 0.26 | 0.22 |
| 1,1-Dichloroethane | 4000 | 0.17 | 0.13 |
| 1,1-Dichloroethane | 600 | 0.14 | |
| 1,1-Dichloroethane | Toluene | 0.095 | |

[a] Solutes, except for toluene, are polystyrenes of the molecular weights noted in the entry. The final column allows for the adsorption term, $C^s_{ads}$ = 0.4, obtained earlier for 4000 mol wt polystyrene in the two-liquid solvents.

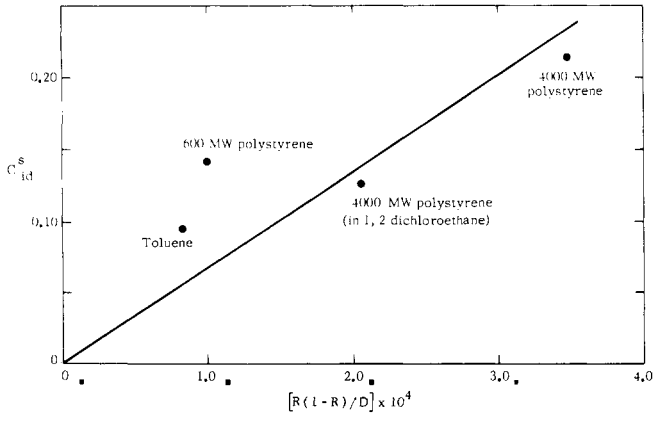


**Figure 5.** Plot of $C_{id}^s$ values from Table III vs. $R(1-R)/D$. The solvent is 1,1-dichloroethane except as otherwise noted. The slope of the line can be identified with $d_p^2/30\gamma_s$.

column size data indicate a porosity of 0.37 for our EC support. This should give a $\gamma_s$ value ca. 0.5 to 0.6, according to the theory. No allowance is made in the theory for molecular dimensions that approach the diameter of the pore.

Our considerable divergence from the expected $\gamma_s$ value, 0.17 instead of ca. 0.6, may be attributed to some special circumstances related to the small pores, to experimental uncertainties, or to some combination of these.

Both random and systematic errors could exist in our data. The particles of our support material, for example, were quite erratic in dimensions; microscopic observations suggest that a value somewhat larger than 59 $\mu$ might serve as a more realistic rms diameter. However, the irregularity of the particles and uncertainty about how to account for shape differences made it difficult to obtain a more meaningful value. Particle diameter is especially important because the calculated $\gamma_s$ depends on the square of the value used.

The above discussion shows that the study of $\gamma_s$ in EC is subject to considerable uncertainty. We believe that EC data measured carefully on columns of uniform, symmetrical particles, and used according to the procedures outlined here, could contribute substantially to the evaluation of $\gamma_s$ terms. These terms are clearly of major importance in EC because the large increments in plate height between permeating and excluded solutes (Figure 3) are directly proportional to $\gamma_s^{-1}$.

## Conclusion

In summary, the foregoing work outlines procedures by which major contributions to plate height can be identified. With these procedures, the effects of solvent, support material, surface treatment, porosity, pore size distribution, and particle size on plate height can be traced to their origins. This should ultimately make it possible to identify the practical barriers to improved EC performance and to find new pathways for improving that performance.

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