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A Review of Peak Broadening in Gel Chromatography

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Summary

Current theories of peak broadening in gel permeation chromatography are discussed in detail. Factors contributing to dispersion in liquid chromatographic systems are reviewed with regard to their significance in GPC. Published data on column efficiency obtained with various columns and packings (both GPC and gel filtration) are compared and interpreted using existing theories.

INTRODUCTION

When a monodisperse substance is passed through a chromatographic column, it is eluted with a distribution of retention times. Everyone is familiar with such broadening phenomena, yet the detailed mechanisms responsible are so complex and varied in nature that they are not well understood. Knowledge of the variables affecting broadening in chromatographic and packed-bed processes is essential for the optimization of process conditions, as well as for the design of improved separation systems. Several theories (*1–12*) have been developed predicting broadening behavior based upon idealized macroscopic models. These theories do not imply, however, that the same type of idealized dispersion mechanisms occur on the microscopic scale during the passage of individual molecules through a packed column. Rather, they are mathematical models of idealized physical processes that allow us to predict broadening behavior.

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A chromatographic separation process can be considered to occur in a series of hypothetical steps, within each of which equilibrium is achieved between solute concentrations in the mobile and stationary phases. Each step is termed a plate and is considered to correspond to a specific height of the column. Although the actual separation does not occur in this manner, the concept of height equivalent to a theoretical plate (H) has proved to be extremely useful for characterizing column efficiency.

The number of theoretical plates, N, can be easily calculated from the chromatogram of a monodisperse substance as

$$N = (T_r/\sigma)^2 \tag{1}$$

where T_r is the mean retention time and σ is the standard deviation. The number of theoretical plates, N, is mathematically defined in terms of the chromatogram (concentration–time distribution), since the retention time, T_r, is the first moment of the distribution and the variance, σ^2, is the second moment of the distribution taken about the mean. In practice, both T_r and σ^2 should be corrected for the finite time and width associated with the sample injection. Once N has been determined, the plate height H may be obtained from

$$H = L/N \tag{2}$$

where L is the column length.

Because of the mathematical nature of the plate height definitions, most of the theoretical expressions developed to predict broadening have utilized this concept. In general, plate height H is predicted in terms of operating variables such as the average carrier (solvent) interstitial velocity (U), the solute molecular diffusivity D_m, and column geometry factors such as packing particle size, column radius, and column length. When correlating plate height data, it is often convenient to define a reduced plate height h and a reduced velocity v according to the following relationships:

$$h = H/d_p \tag{3}$$

$$v = Ud_p/D_m \tag{4}$$

where d_p is the effective particle diameter. It should be noted that the reduced velocity is analogous to the product of the Reynolds number times the Schmidt number and is also a Peclet number based on the molecular diffusivity. The utility of these relationships will become apparent later in the paper.

Phenomena such as eddy diffusion, molecular diffusion, velocity-profile effects (or nonequilibrium effects) in the mobile phase, dead-volume effects, sorptive effects, viscosity effects (such as viscous fingering), and dispersion due to diffusion into and out of the pores (the permeation process) may be encountered to varying degrees in separation processes such as gel permeation chromatography (GPC) or gel filtration (*9*). Broadening can be further separated into mobile-phase dispersion occurring in the absence of permeation or adsorption, and broadening associated with the separation mass-transfer process itself. It is important to understand the dispersion contributions occurring in the mobile phase (in the absence of mass transfer) before attempting to interpret the overall dispersion present in actual chromatographic systems.

There are often objections to the use of plate height since it depends upon so many variables. In another approach, Hendrickson (*13*) postulated that peak spreading obeys an equation of the form

$$\bar{W}_b^2 = \bar{W}_m^2 + \bar{W}_a^2 + \bar{W}_i^2 + \bar{W}_d^2 + \bar{W}_s^2 \qquad (5)$$

where $\bar{W}_b$ is the observed width (at the base) of the chromatogram, and the other terms represent, in order, the contributions to that width from the molecular-size distribution of the test sample, the apparatus, spreading in the interstitial volume within the column, diffusional spreading due to holdup of molecules within the pores of the gel, and sorption.

Excellent reviews of GPC and gel filtration have been presented by Altgelt and Moore (*14*), Altgelt (*15*), and Determan (*16*), but no comprehensive review correlating broadening theory and experimental data has appeared. It is the purpose of this paper to discuss current theories of peak broadening with respect to published data on column efficiency obtained with various columns and packings. Mobile-phase dispersion effects will be reviewed to provide a background for understanding broadening behavior in gel chromatography. Factors contributing to peak broadening in liquid chromatographic systems will be reviewed with regard to their significance in gel chromatography.

THEORY

In general, plate height H may be related by the equation:

$$H = 2D/U + (\text{a mass transfer contribution}) \qquad (6)$$

where D is an overall dispersion number. Utilizing this equation, van

Deemter (*1*) assumed that the dispersion number was composed of a longitudinal molecular-diffusion term and an eddy-diffusion term, and that the mass-transfer equation was a linear function of carrier velocity:

$$D = \underbrace{\phi D_m}_{\substack{\text{molecular} \\ \text{diffusion}}} + \underbrace{\lambda U d_p}_{\substack{\text{eddy} \\ \text{diffusion}}} \tag{7}$$

where ϕ is a tortuosity factor ($\phi = 2/3$) and λ is an eddy-diffusion proportionality factor ($\lambda \approx 1/11$). This resulted in an overall equation for plate height of the general form

$$H = A + (B/U) + CU \tag{8}$$

where the first term accounts for eddy diffusion, the second term for molecular diffusion, and the third term for mass-transfer resistances in the stationary and mobile phases. In the absence of mass-transfer processes, and with appropriate substitutions, Eq. (8) becomes

$$H = 2\lambda d_p + 2\phi D_m/U \tag{9}$$

which applies to mobile-phase dispersion effects.

For some chromatographic systems, however, van Deemter's approach did not correlate well with experimental results. Perhaps the most important reason for lack of correlation was the difficulty in accounting adequately for the complex velocity and flow profiles existing within a packed column. The concept of eddy diffusion is highly idealized and assumes that particles remain fixed in stream lines, that stream lines split when they impinge directly on a particle, and that perfect mixing occurs between particles when stream lines combine. In addition, this approach did not account adequately for variations in aspect ratio (δ = column diameter/particle diameter) and variations in packing uniformity. Further objections arose to the form of the mass-transfer contribution to plate height. It was to explain these deviations between theory and experiments that Giddings (*2–4*) developed his "coupling theory." In this theory, the eddy diffusivity is coupled in a nonadditive manner with the mobile-phase resistance to nonequilibrium mass transfer. In general form, the coupling equation theory of Giddings may be written

$$H = (B/U) + C_s U + \sum_i \frac{1}{(1/A_i) + (1/C_{mi}U)} \tag{10}$$

where U is the carrier velocity, B takes into account molecular diffusion, C_s accounts for mass-transfer effects in the stationary phase, A_i accounts for eddy diffusion, and C_{mi} takes into account mass-transfer effects in the mobile phase. For describing mobile-phase dispersion in the absence of mass transfer between a stationary and a mobile phase, Giddings' coupling theory predicts

$$H = \frac{2\gamma D_m}{U} + \sum_i \frac{1}{(1/2\lambda_i d_p) + (D_m/\omega_i d_p^2 U)} \tag{11}$$

where γ, λ_i, and ω_i are geometrical constants. In terms of the reduced parameters h and v, Eq. (11) becomes

$$h = 2\gamma/v + \sum_i \frac{1}{(1/2\lambda_i) + (1/\omega_i v)} \tag{12}$$

Giddings and Mallik (7) applied Eq. (10) specifically to the theory of zone broadening in gel filtration (permeation) chromatography, defining the parameters A_i, B, C_s, and C_{mi} in the context of the GPC process. Because of the obstructions to diffusion posed by the gel network, the diffusion coefficient for the solute in the mobile phase (D_m) differs from that in the stationary phase (D_s). An obstruction factor for the gel was defined as $r = D_s/D_m$ and was estimated to be approximately equal to 2/3. A reduced velocity (R) was defined as the ratio of the zone velocity to the mobile-phase velocity. The final form of the equation proposed for plate height is:

$$H = \frac{4}{3}\frac{D_m}{RU} + \frac{1}{20}R(1 - R)\frac{d_p^2 U}{D_m} + \sum_i \frac{1}{(1/2\lambda_i d_p) + (D_m/\omega_i d_p^2 U)} \tag{13}$$

where λ_i and ω_i are geometrical factors of order unity.

For gel chromatography, the variation of reduced plate height with reduced velocity according to Eq. (13) is shown in Fig. 1. The curve is concave downward at low flow velocities, becoming linear at higher reduced velocities. The magnitude of the plate height predicted is quite dependent upon the evaluation of the coupling term.

Another approach (7–9) which helps to explain the origin of coupling has been to extend van Deemter's theory by incorporating an additional term to account for velocity-profile effects caused by a nonuniform velocity over the column cross section. A nonuniform velocity profile causes a spread in retention times whose magnitude is deter-

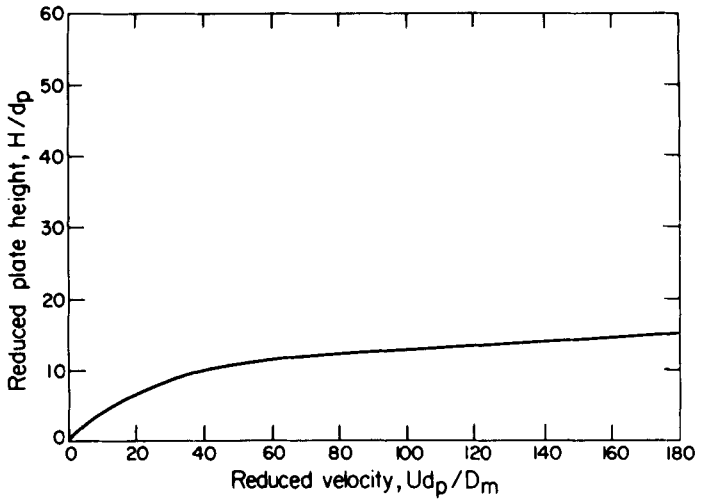

FIG. 1. Effect of reduced velocity on reduced plate height according to the theory of Giddings and Mallik (7).

mined primarily by radial (transverse) diffusion. Johnson (*12*) applied this approach to gaseous systems and stated that a major portion of the observed broadening in liquid systems results from velocity-profile effects. Sie and Rijnders (*6*) found this approach valuable for describing band broadening in packed chromatographic columns for gaseous systems, and pointed out that it may also be useful in liquid chromatography. We have previously indicated (*8, 9, 17*) the value of utilizing a velocity-profile approach for describing dispersion effects occurring in the mobile phase and of its incorporation into an overall model for describing broadening in GPC and gel filtration (*8, 18*).

Most chromatographic column-packing materials have a relatively wide particle-size range. During column packing, the particles can segregate, producing some cross sections having a small-particle-diameter packing and others having large-diameter packing. Such particle-size segregation, coupled with variations in packing density, presents variable resistance to the flowing fluid and leads to a nonuniform velocity profile. Because the column void fraction is greater near the wall, the flowing fluid encounters less resistance there, and the average velocity near the wall is correspondingly greater than in the center of the bed. Several investigators have reported this behavior (*12, 19–22*).

The overall longitudinal-dispersion number D is therefore assumed to be the sum of contributions from molecular diffusion, eddy diffusion, and velocity-profile effects operating in the column:

$$D = \underbrace{\phi D_m}_{\substack{\text{molecular}\\ \text{diffusion}}} + \underbrace{\lambda U d_p}_{\substack{\text{eddy}\\ \text{diffusion}}} + \underbrace{\hbar R_c^2 U^2/\bar{D}_r}_{\substack{\text{velocity-profile}\\ \text{effects}}} \quad (14)$$

where $\hbar$ is a velocity-profile constant, R_c is the column radius, and $\bar{D}_r$ is an average radial diffusivity. This form of the expression for the overall dispersion number was developed by Taylor (*23*) and Aris (*24*).

In cases where a mass-transfer process such as adsorption or permeation is absent or negligible, the plate-height equation becomes:

$$H = (2\phi D_m/U) + 2\lambda d_p + (2\hbar R_c^2 U/\bar{D}_r) \quad (15)$$

The utility of this equation for describing mobile-phase dispersion in actual liquid chromatographic systems depends greatly on the evaluation of the average radial diffusivity $\bar{D}_r$ and the appropriate velocity-profile constant $\hbar$ for the experimental column used. Previous work (*6*, *9*, *25–27*) has shown that the radial diffusivity is determined by radial gradients existing within the column which come about from both molecular-diffusion and eddy-diffusion processes. These in turn depend upon the variation of velocity across the column:

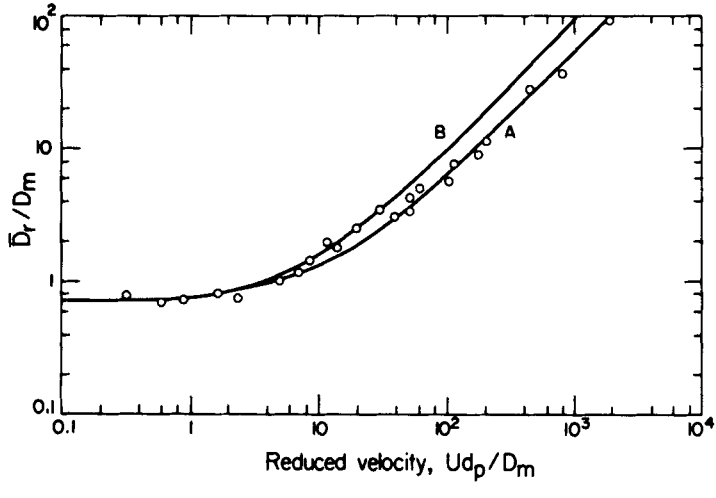

FIG. 2. Theoretical and experimental radial dispersion coefficients for randomly packed beds of uniform-size beads. Curve A was calculated using the equation $\bar{D}_r/D_m = 2/3 + 0.091\ Ud_p/D_m$. Curve B was calculated using the equation $\bar{D}_r/D_m = 0.7 + 0.055\ Ud_p/D_m$. Experimental data were taken from Grane and Gardner (*28*) and Blackwell (*29*). This figure was, in part, redrawn from Perkins and Johnston (*25*).

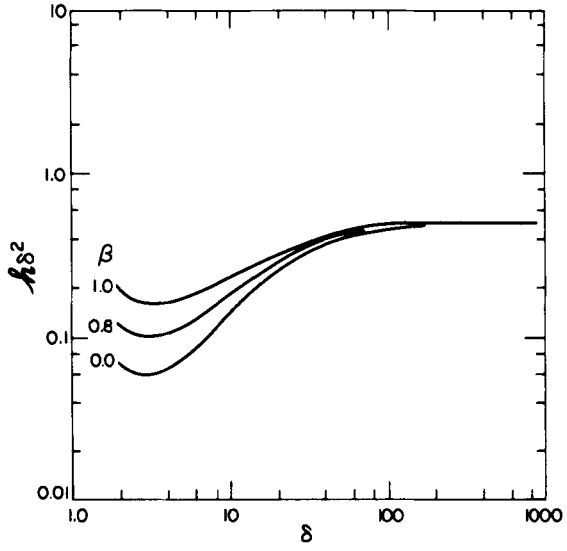

FIG. 3. Effect of aspect ratio (δ) on the dispersion parameter ($\hbar\delta^2$) for packed beds of uniform-size beads. Figure redrawn from Johnson (*12*). β = a dispersion intensity ratio $= \lambda U d_p/(\lambda U d_p + \phi D_m)$.

$$D_r(\rho) = \phi D_m + \lambda d_p U(\rho) \tag{16}$$

where ρ is a dimensionless radial position variable: $\rho = r/R_c$, where r is the actual radial position variable. From Eq. (16), the average radial diffusivity is:

$$\bar{D}_r = \phi D_m + \lambda d_p U \tag{17}$$

Figure 2 shows that Eq. (17) gives excellent agreement with experimental data from the literature, and therefore the average radial diffusivity may be predicted accurately.

Perhaps the biggest drawback for utilizing the velocity-profile model in the past has been the difficulty in evaluating the velocity-profile constant $\hbar$. Johnson (*12*) has rigorously evaluated $\hbar$ for packed-bed systems as a function of aspect ratio and β (which is a measure of the relative importance of eddy diffusion versus the combination of eddy and molecular diffusion). As a result of Johnson's work, it is now possible to determine graphically with the aid of Fig. 3 (*12*) the velocity-profile constant for the particular column system of interest (*9*, *12*). The calculations with the velocity-profile model then become relatively simple.

Incorporating these concepts, the plate height equation describing *mobile-phase dispersion* with a velocity-profile model becomes:

$$H = 2[(\phi D_m/U) + \lambda d_p] + 2\hbar R_c^2/[(\phi D_m/U) + \lambda d_p] \quad (18)$$

It is obvious that the denominator of the second term of Eq. (18) represents a "coupling" of the eddy and molecular diffusivities, similar to that of Giddings. From the form of Eq. (18) it appears that for a given particle diameter the contribution to plate height from the velocity-profile term increases with the square of the column radius. This is not so, since the last term of Eq. (18) may be rewritten

$$2\hbar R_c^2/[(\phi D_m/U) + \lambda d_p] = \hbar\delta^2 d_p^2/2[(\phi D_m/U) + \lambda d_p] \quad (19$$

At constant d_p this contribution to plate height varies linearly with the dispersion parameter $\hbar\delta^2$ which is nearly constant at aspect ratios above 40, the region of practical importance.

Kelley and Billmeyer (*18*) recently proposed that the effect of increased broadening in GPC and gel filtration due to the permeation of solute molecules into and out of the pores may be diffusion controlled. To account for such broadening, a term which is linear with carrier velocity is added to Eq. (18) to provide an overall equation for plate height in GPC:

$$H = 2[(\phi D_m/U) + \lambda d_p] + 2\hbar R_c^2/[(\phi D_m/U) + \lambda d_p] + CU \quad (20)$$

A schematic representation of the relative importance of the terms in this equation is given in Fig. 4.

Huber (*10, 11*) has recently developed a theory to describe broadening in liquid chromatographic systems. The overall expression for plate height is expressed as a sum of individual contributions which include

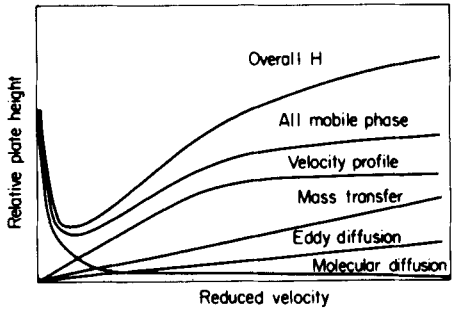

FIG. 4. Schematic representation of the relative contributions to plate height with a velocity-profile model.

mixing arising from diffusion in the mobile phase (H_{md}), mixing due to convection in the mobile phase (H_{mc}), and contributions from mass-transfer effects in the mobile (H_{em}) and in the stationary phases (H_{es}). These effects were assumed to be independent and additive quantities (as has been assumed in previous theories) such that

$$H = H_{md} + H_{mc} + H_{em} + H_{es} \tag{21}$$

where

$$H_{md} = (2\epsilon_m/\tau_m)(D_{ia}/U) \tag{22}$$

$$H_{mc} = 2\lambda d_p/\{1 + \lambda_2(D_{ia}/Ud_p)^{1/2}\} \tag{23}$$

$$H_{em} = (1/5.7)\left(\frac{\epsilon_a - \epsilon_m + K_i\epsilon_\beta}{\epsilon_a + K_i\epsilon_\beta}\right)^2 \frac{\epsilon_m^{1/2} d_p^{3/2} \nu_\alpha^{1/6} U_{ia}^{2/3}}{(1 - \epsilon_m) D_{ia}^{1/2}} \tag{24}$$

$$H_{es} = (1/30)\frac{\epsilon_a - \epsilon_m + K_i\epsilon_\beta}{(\epsilon_a + K_i\epsilon_\beta)^2} \frac{\epsilon_m(1 - \epsilon_m)\tau_s}{\epsilon_s} \frac{d_p^2 U}{D_{is}} \tag{25}$$

ϵ_m = the interstitial volume fraction
ϵ_s = the internal pore volume fraction occupied by stationary fluid
ϵ_a = volume fraction occupied by the moving phase
ϵ_β = volume fraction occupied by the stationary phase
λ_1 and λ_2 = geometric constants
τ_m, τ_s = tortuosity factors
K_i = distribution coefficient equal to the ratio of the equilibrium concentrations in the stationary and mobile phases
ν_α = the kinematic viscosity of the mobile phase
D_{ia} = the diffusion coefficient in the mobile phase
D_{is} = the diffusion coefficient in the stationary phase

Huber's approach takes into account the specific volume fractions in which the broadening processes are occurring. The overall plate height curve as a function of fluid velocity is concave downward with decreasing flow rate until molecular diffusion effects begin to dominate where a sharp rise in H is observed. The effect of H_{md} is less important at high flow rates. Contributions due to H_{em} and H_{mc} are both concave downward with decreasing flow rate, with the larger contribution coming from H_{mc}. The term H_{es} is linear with flow rate. The overall plate height behavior is qualitatively very similar to that of Giddings (*7*) and Kelley and Billmeyer (*9, 18*).

Broadening occurring in the mobile phase results by definition from all dispersion processes that affect the residence time distribution but

in which the solute molecules are not removed from the mobile phase. Adsorption and permeation are mass transfer processes which involve a stationary phase in which the solute molecules become temporarily entrapped during their passage through the column. Major sources of mobile phase dispersion included in the theoretical models just discussed are molecular diffusion, eddy diffusion, and nonequilibrium effects associated with velocity variations due to packing geometry. Dispersion associated with stagnant regions, boundary-layer phenomena, natural convection effects resulting from density gradients, viscous fingering, and other concentration effects have not been incorporated into the mathematical dispersion models. All of these effects could be present to varying degrees in an actual column system and would lead to disagreement between theory and data. It is probable that stagnant regions or "dead volume" areas do exist in real systems. Transfer into and out of such dead volume is diffusion controlled and would give a linear contribution to plate height. At flow rates normally encountered in gel chromatography, boundary layers developed around the particles should be very thin and would not appreciably affect the observed dispersion. Grashoff numbers (measures of natural convection) are estimated to be well below 1.0 for gel chromatography at normal flow rates, indicating that natural convection should not be important. The low Grashoff numbers result from the generally small particle sizes employed, together with the very low solute concentrations.

MOBILE-PHASE DISPERSION IN NONPOROUS SYSTEMS

Many excellent reviews of mobile-phase dispersion phenomena in gaseous (*4, 12, 30, 31*) and liquid (*32–35*) systems have been published. There is, however, a need for further research into the basic nature of dispersion occurring under conditions such as those encountered in the liquid chromatography of macromolecules. The following discussion deals only with dispersion data applicable to gel chromatography.

1. Extra-Column Effects

Before accurate dispersion measurements can be made with packed columns, the extra-column broadening associated with the sample injection and detection system must be evaluated and minimized. To evaluate these contributions to peak broadening, samples are usually injected through the sample loop directly into the detector with no column in the system. Billmeyer and Kelley (*17*) noted considerable

tailing (asymmetry) and other anomalies in the injection-detection pattern of samples of macromolecular solutes utilizing the Waters Model 100 Gel Permeation Chromatograph with a standard refractometer cell (volume 70 μl) and a 1/16-in. null glass. Similar effects have been noted by other investigators (*10, 36–38*).

Huber and Hulsman (*10*) utilized a modified flow arrangement with a Waters type R4 differential refractometer containing a micro refractometer cell having a volume of 10 μl to reduce mixing in the apparatus outside the column during the liquid chromatography of small molecules. Billmeyer and Kelley (*17*) minimized extra-column broadening and associated flow anomalies by changing to a micro refractometer cell, minimizing the amount of tubing in the system (and eliminating the heat exchanger), using short injection times, and reducing the solute concentration. By making the above modifications, the extra-column contributions to broadening were made practically negligible compared to the broadening occurring during passage through a single GPC column.

Carmichael (*39*) has formally evaluated the extra-column and column contributions to the chromatogram. Through knowledge of the individual extra-column and column effects associated with monodisperse substances, the column contribution to the elution curve for an arbitrary molecular size distribution can be determined. The individual column contributions can be mathematically evaluated for monodisperse substances by using a stochastic model for gel chromatography (*40–42*) and assuming a Gaussian distribution (*43*) for the chromatogram. Extra-column effects are separately evaluated for the system without the column.

2. Diffusivity and Flow Rate Effects

Axial dispersion of molecules during flow through packed beds, such as chromatographic columns, is known to be greatly dependent upon the amount of radial mixing. The importance of radial processes necessitates further discussion of the mechanisms involved. In Fig. 2, radial dispersion was shown to be due to the combination of molecular diffusion and eddy diffusion. Fickian molecular diffusion due to a concentration gradient, occurring in both the radial and axial directions, is a well-known concept. Molecular diffusion becomes extremely important at very low flow rates. Eddy, or as it is sometimes referred to, convective diffusion, is lateral transport caused by "stream splitting." A stream line impinging upon a particle cannot go through,

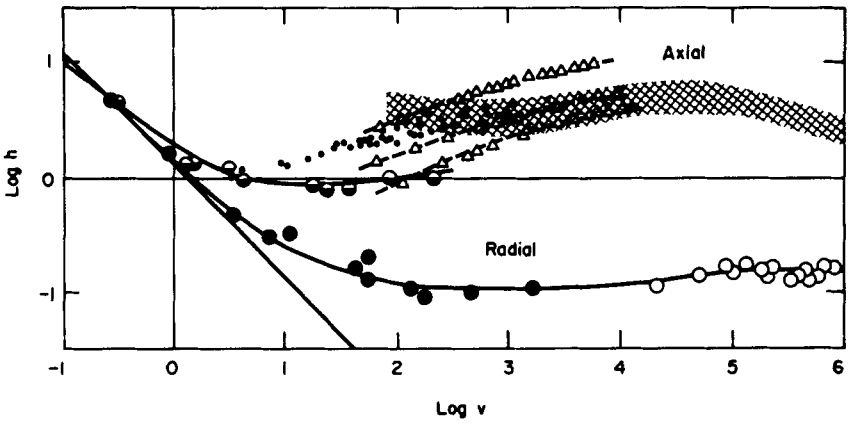

FIG. 5. Variation of axial and radial reduced plate height as a function of reduced velocity. Symbols: ○, Bernard and Wilhelm (*45*); ●, Blackwell (*29*); ◒, Brigham, Reed, and Dew (*46*); ●, Kelley and Billmeyer (*9, 47*), data for 120–140 mesh glass bead column with solutes of varying diffusivity; △, Knox (*48*). Cross-hatched area represents the range of data of Ebach and White (*49*), a total of 50 data points.

so it splits, with part of the original stream line going one direction and the other part in the opposite direction. By this idealized process the original stream line is displaced laterally by one-half the particle diameter. Eddy diffusion predominates at high flow rates. Sie and Rijnders (*6*) have explained the mechanism of eddy diffusion in detail and have pointed out that the magnitudes of the eddy and molecular diffusivities are often nearly equivalent in liquid chromatography. Hiby (*44*) has experimentally demonstrated stream splitting by following stream lines of dye in an idealized two-dimensional bed of spheres.

Axial and radial dispersion data of several investigators (*9, 29, 45–49*) are summarized in Fig. 5. Radial plate heights are considerably lower than those observed from axial dispersion except at low reduced velocities where molecular diffusion dominates. (A range of reduced velocity between 10 and 10^4 is commonly encountered in the gel chromathography of macromolecules.) Axial dispersion results (*9, 47*), obtained from measurements utilizing high molecular weight solutes and nonporous glass bead GPC columns show good agreement with literature data.

The effect of molecular diffusivity on the resulting band broadening as a function of flow rate (expressed in terms of Reynolds number) is illustrated in Fig. 6. The diffusivities cover a wide range, from approxi-

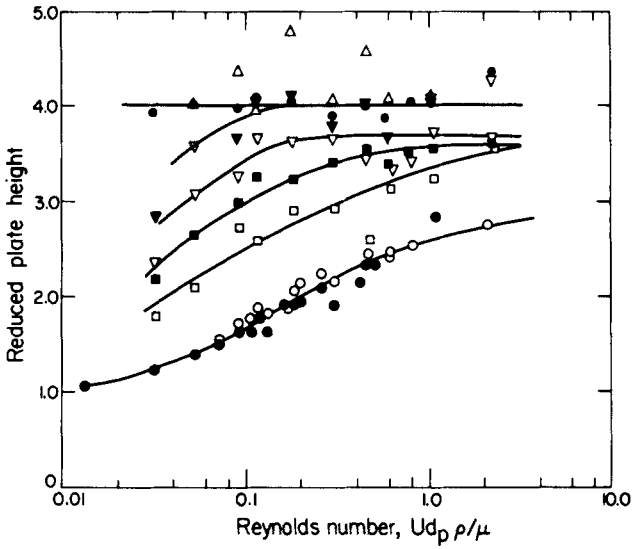

FIG. 6. Reduced plate height vs Reynolds number for 120–140 mesh nonporous glass bead column. ●, Hexane; ○, cyclohexane; □, n-$C_{36}H_{74}$; ■, 2,000 PS; ▽, 3,600 PS; ▼, 10,300 PS; ⊕, 19,800 PS; △, 97,200 PS. Toluene solvent at room temperature. Data of Kelley and Billmeyer (*9*, *47*).

mately 2×10^{-5} cm^2/sec for hexane to 5×10^{-7} cm^2/sec for the 97,200 molecular weight polystyrene standard. Plate height curves for low molecular weight materials are concave downward with decreasing flow rate, while dispersion is relatively unaffected by flow rate for high molecular weight substances such as 97,200 polystyrene. Similar concave downward curvature has been noted by Huber (*11*) for small solute molecules with inert diatomaceous earth packings. Qualitatively, this dependence of broadening on diffusivity is predicted theoretically by the models of Giddings (*7*) and Huber (*10*, *11*), and by the velocity-profile model (*8*, *9*). It is not consistent, however, with van Deemter's original theory (*1*).

Horne, Knox, and McLaren (*34*) stated that the major factor producing dispersion between the flow region where molecular diffusion dominates and that where turbulence dominates is the slow rate at which transcolumn equilibrium is achieved. Kelley and Billmeyer (*9*) found that the major source of mobile phase dispersion was the velocity-profile term which includes the radial diffusivity in the denominator.

Dispersion caused by lack of equilibrium or velocity-profile effects usually increases with decreasing radial diffusion. This term becomes especially important with high molecular weight solutes having very low molecular diffusivities.

3. Aspect Ratio and Particle Size Distribution Effects

The aspect ratio, or the column diameter divided by the particle diameter, is very important in determining the magnitude of wall, velocity-profile, or transcolumn nonequilibrium effects. Experimentally, aspect ratio dependence may be investigated either by changing the particle diameter at constant column diameter or by changing the column diameter for a given packing. Both procedures have limitations. Changing the particle diameter may change the particle size distribution (when nonuniform diameter materials are employed) by introducing an additional variable. Changing the column radius may lead to additional dispersion associated with entrance and exit effects. Column packing uniformity is generally difficult to repeat experimentally since it is very dependent upon the packing techniques employed.

Particle size variations in a given packing material are known to cause segregation during packing which leads to increased dispersion in gaseous systems. Such dependence of dispersion on the breadth of the particle-size distribution has not been extensively investigated in liquid chromatography until recently.

Kelley and Billmeyer (*9*) found that decreasing the particle size leads to improved column efficiency, as shown in the plate height curves in Fig. 7. Smaller particle sizes both reduce the eddy-diffusion contribution to broadening and lead to reduced wall effects associated with the larger aspect ratios. Such behavior is confirmed by experimental results of Huber (*11*). At very high aspect ratios (greater than 100), theory predicts that the dispersion should be relatively insensitive to further increases in aspect ratio. Most gel chromatography systems employ aspect ratios over 100, as in GPC with standard column diameters of 0.775 cm and particle diameters of less than 40 μ.

Knox and Parcher (*27*) examined the effects of aspect ratio on mobile-phase dispersion in liquid chromatography. Most of the data were obtained at low aspect ratios to determine dispersion variations caused by wall effects and associated transcolumn nonequilibrium. Giddings' nonequilibrium theory (*2–4*) was extended to include the effects of aspect ratio and varying velocity profile. At high aspect

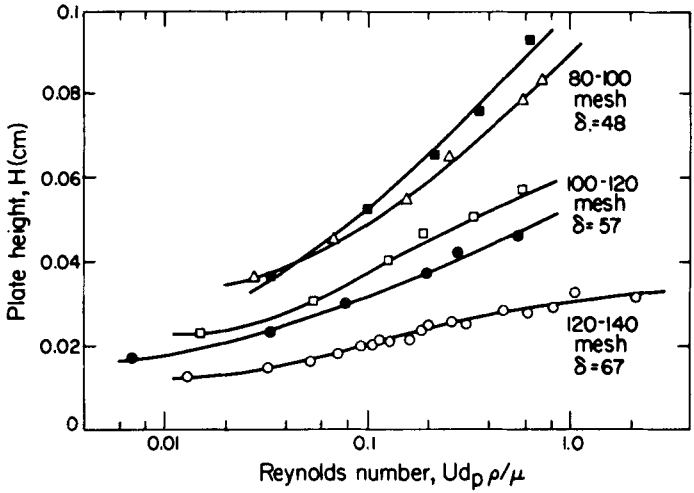

FIG. 7. Effects of particle size and segregation on dispersion of cyclohexane. Conditions same as in Fig. 6. ○, 120–140 mesh; □, 100–120 mesh; △, 80–100 mesh; ■, 1/1 by volume 80–100 mesh/100–120 mesh; ●, 1/1/1 by volume 80-100 mesh/100-120 mesh/120-140 mesh.

ratios (about 10), the trans-column contribution to plate height was found to become independent of column diameter. A sharp increase in plate height was observed in the range $6 < \delta < 8$.

Horne, Knox, and MaLaren (*34*) examined the importance of particle-size distribution on plate height and found only slight increases in broadening as the distribution was increased. Kelley and Billmeyer (*9*) reexamined the importance of the particle-size distribution utilizing higher aspect ratios, as shown in Fig. 7, and found that the breadth of the distribution did not alter the resulting dispersion significantly (with calculations carried out at the average particle size for the distribution). These results indicate that the breadth of the particle-size distribution is relatively unimportant for mobile phase dispersion in liquid systems. This is not true with gaseous systems. Further research is necessary to explain such differences between gaseous and liquid systems.

4. Discussion

The broadening models proposed by Giddings (*2–4*) and Huber (*10, 11*), and the velocity-profile model adapted to liquid systems by Billmeyer and Kelley (*8, 9*) seem to predict the observed trends

qualitatively for a wide range of variables. These theories may be utilized to assess the relative importance of operational variables on mobile phase dispersion in gel chromatography. Before their relative merits for predicting dispersion quantitatively can be determined, more extensive experimental data are required. It is clear, however, that the experimental results do not correlate with van Deemter's Eq. (1). It is also apparent that the major source of dispersion is often trans-column nonequilibrium effects associated with the column walls and the velocity profile across the bed.

BROADENING IN POROUS SYSTEMS

The broadening effect caused by a mass-transfer process such as adsorption or permeation can often be accounted for by an additional term in the plate-height equation. In the case of gas chromatography, it has been established (*1*) that the variation of plate height due to the sorption-desorption process is linear with gas velocity. Such a linear and additive term arises, in part, from a linear adsorption isotherm.

Kelley and Billmeyer (*18*) studied peak broadening in gel chromatography with nonporous and porous column packings using identical operation conditions. The difference between the results, that is, the contribution to plate height due to permeation into and out of the pores, was a linear function of flow velocity. Such a linear dependence suggests that the broadening associated with permeation may be diffusion controlled.

Variations in plate height were found to be dependent upon the particular gel chromatography packing material employed (*18*). For a porous glass (Porasil) packing, dispersion arising from permeation was the major contribution to peak broadening, whereas for a poly-styrene-divinylbenzene) (Styragel) packing, dispersion due to mobile-phase effects was predominant. In order to explain these results qualitatively, it is necessary to study the differences in pore structure of the two materials.

For convenience, broadening in gel chromatography will be discussed separately according to the porous packing employed. The packing material most commonly used in analytical GPC is cross-linked poly(styrene-divinylbenzene) manufactured and marketed by Waters Associates under the trade name Styragel. Cross-linked porous dextran gels (Sephadex, Pharmacia Fine Chemicals Inc.) are widely used in biological applications for separations described as gel filtration. The properties of these gels have been described by Altgelt

and Moore (*14*). In addition, recently-developed porous glass beads are finding many applications in gel chromatography, especially where chemical inertness and structural stability are prime considerations in selecting the column packing.

Three different types of porous glass beads have been developed for gel chromatography. LePage and de Vries (*38*, *50–52*) reported the use of porous silica beads which are manufactured by Pechiney-Saint-Gobain (*53*) and currently distributed by Waters Associates under the trade name Porasil. Haller (*54–56*) developed a different process for producing porous silica beads now made by the Corning Glass Co., with Waters Associates as distributors. Another type of glass beads with the trade name Bio-Glas, having controlled pore size, has been developed by Bio-Rad Laboratories of Richmond, California (*57*). They also produce porous polyacrylamide gels, agarose gels, and poly(styrene-divinylbenzene) beads for gel chromatography. The structure of Bio-Glas has been described by Barrall and Cain (*58*) and its properties as a packing material for GPC have been discussed by Cantow and Johnson (*59*, *60*). See also Cooper et al. (*60a*).

1. Gel Filtration

Flodin (*61*) examined the effect of flow rate and particle size on plate height in gel chromatography with Sephadex G-25 gel. Data indicate that as the flow rate was increased, plate height also increased from 0.39 mm at 0.053 ml/cm^2-min to 5.49 mm at 1.01 ml/cm^2-min with uridylic acid as the solute. The increase probably resulted primarily from the increasing importance of nonequilibrium effects at higher flow rates. A decrease in plate height from 1.05 mm at 0.074 ml/cm^2-min to 0.51 mm at 0.51 ml/cm^2-min was noted for hydrochloric acid solute with increased flow rate. The higher plate height at the lower flow rate resulted from axial molecular diffusion due to the higher diffusion coefficient for hydrochloric acid solute. Higher plate heights were associated with higher particle sizes.

Giddings and Mallik (*7*) tabulated plate height results from gel filtration data of Porath (*62*, *63*) with Sephadex gel under varying operating conditions. Reduced plate heights were generally above 10 at reduced velocities lower than 50. The high values of plate height and scatter in the data may have resulted from extra-column effects, microscopic packing variations, and particle-size effects. It was concluded that the contribution to plate height from the stationary phase term was relatively small, since stationary-phase diffusion was not rate-controlling.

Edwards (*64*) applied a reaction kinetic model for fixed-bed processes to gel-chromatography data of Flodin (*61*). By calculating the distribution coefficients and overall mass-transfer coefficient from the various resistances, the height of a reaction unit together with the number of reaction units could be obtained. Relatively close agreement between the predicted (855) and the observed (653) number of theoretical plates was obtained with sodium chloride solute. However, predicted values for hemoglobin (1630 plates) gave poor agreement with observation (625 plates). This lack of agreement was thought to result from a viscous effect, and the fact that the equations were developed for low molecular weight compounds and may have required modification to account for the larger axial dispersion coefficients of macromolecules.

2. Poly(styrene–divinylbenzene) Gels

Plate-height data of several investigators (*8*, *13*, *65*, *66*) utilizing Styragel columns are summarized in Fig. 8. Plate heights increase with increasing solvent flow rate. Generally, the curves are concave downward with decreasing flow rate.

Giddings and Mallik (*7*) reviewed the concave downward plate-height data of Smith and Kollmansberger (*65*), concluding that they are in qualitative agreement with the coupling theory expressed in Eq. (10) and shown in Fig. 1. Such a concave-downward relationship is predicted by the theories of Giddings (*7*), Huber (*10*, *11*), and Billmeyer and co-workers (*8*, *18*), but not by that of van Deemter et al. (*1*).

Heitz (*67–69*) investigated the variation of plate height with reduced velocity for poly(styrene-divinylbenzene) gels of varying cross-linking density. Separation efficiency was lowered with decreasing cross-linking density. At low reduced velocities (<2), plate height increases due to axial molecular diffusion were observed. Deviations from broadening theory were found to be caused by an alteration of the diffusion coefficient in the stationary phase with respect to that in the mobile phase.

Recently, Little et al. (*70*) examined the broadening behavior of Styragel columns for fast GPC at flow rates near 10 ml/min. Over the flow rate range from 0.1 to 12.5 ml/min, elution volume was found to be independent of flow rate for both small and large molecules. The flow rate dependence of peak width was found to be significantly lower than predicted by the van Deemter equation. Peak width was found

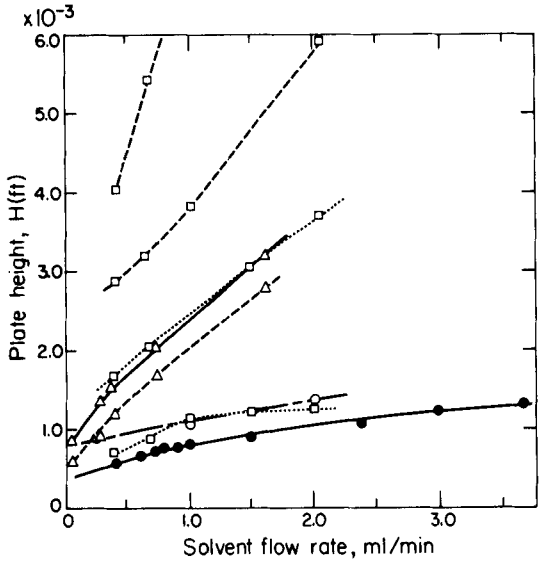

FIG. 8. Comparison of plate height data obtained on Styragel columns. ●, Billmeyer et al. (*8*); △, Smith and Kollmansberger (*65*); □, Hendrickson (*13*); ○, Duerksen and Hamielec (*66*).

generally to be independent of solute concentration (over a range from 0.05 to 0.5% for small molecules), suggesting that "viscous fingering" effects were minimized by the increased mixing (shear) associated with high velocities. Peak symmetry also increased with increasing flow rate.

A marked resemblance exists between the concave-downward plate height curves obtained in gel chromatography with poly(styrene-divinylbenzene) gels and the plate-height relationships observed for mobile phase dispersion using nonporous beads. Giddings and Mallik (*7*) stated that the stationary-phase term in gel permeation should not make a significant contribution to plate height until the reduced velocity becomes considerably greater than 100. High relative velocities can therefore be employed to give more rapid separations. These conclusions are supported by the data of Kelley and Billmeyer (*18*) for Styragel systems.

3. Porous Glass Systems

Reduced plate height data obtained by LePage et al. (*38*) and by Kelley and Billmeyer (*18*, *47*) for Porasil E columns are compared

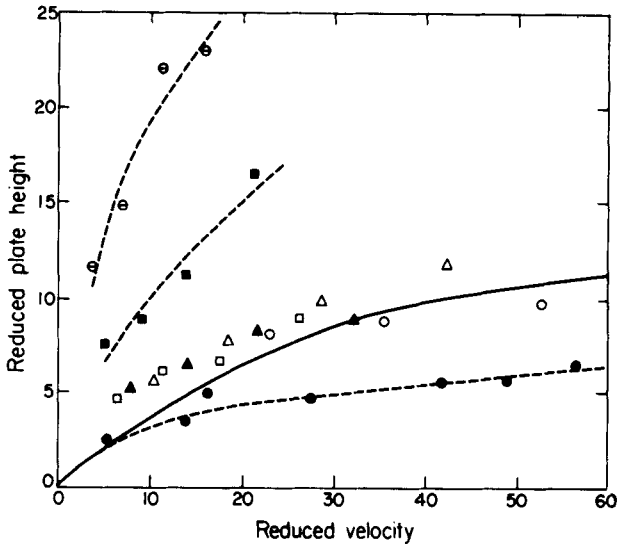

FIG. 9. Comparison of reduced plate height data as a function of reduced velocity for Porasil E. Effect of particle size. Data of LePage et al. (*38*): ⊖, 60–80 μ; ■, 80–100 μ; □, 100–125 μ; ▲, 125–150 μ; △, 160–200 μ; ○, 200–250 μ. Data of Kelley and Billmeyer (*18*, *47*): ●, 100–125 μ.

in Fig. 9. Reduced plate height curves, as a function of reduced velocity, are concave downward and show good agreement between investigators.

When the plate height data of Kelley and Billmeyer (*18*, *47*) are plotted as a function of the log of the Reynolds number, a concave-downward relationship is not obtained. Instead, the resulting curve is concave upward showing very rapid increases in plate height with increasing Reynolds number. This serves to illustrate the point that the same data may convey outwardly two entirely different messages depending upon the plotting technique.

The data of LePage et al. (*38*) in Fig. 9 show that the reduced plate height (at constant reduced velocity) decreases as the particle size increases. Reduced broadening at higher particle sizes is difficult to explain in light of the theories of peak broadening which are supported by the data of Flodin (*61*) for gel filtration, together with the mobile phase data of Knox (*27*, *34*) and Kelley and Billmeyer (*7*). Variation of pore geometry with particle size, differences in packing techniques, combined with the possibility of agglomeration of the finer particles during packing may account for the discrepancy (*70a*).

Dispersion is generally greater for Porasil columns than for Styragel columns at equivalent reduced velocities (*18*) and increases more rapidly with increasing flow rate. Reasons for the increased dispersion have been attributed to internal pore structure differences between Porasil and Styragel (*18*). Essentially, if a molecule can enter a pore in a Porasil bead, it has a greater probability (compared to the case in Styragel) of continuing to diffuse toward the center of the bead before leaving to return to the mobile phase. Such diffusion within the stationary phase leads to increased broadening. We have stated (*18*) that while mobile-phase dispersion effects are predominant in Styragel columns, the stationary-phase effects (which were found to be linear with flow rate) are predominant in the Porasil systems studied. Pore depth generally increases with particle size in porous glass systems. Broadening occurring in the stationary phase may be minimized by reducing the particle diameter. Reduced particle diameters also lead to shorter interparticle distances and therefore more rapid lateral mixing.

Moore and Arrington (*71*) investigated the separation mechanism in GPC with porous glass beads furnished by W. Haller of the National Bureau of Standards. Plate height was concave downward with flow rate. Column efficiencies greater than 1000 plates were attained with 122 cm columns.

Sliemers et al. (*72*) examined factors affecting the efficiency of GPC columns packed with Porasil beads. Column efficiency (as evaluated by plate height) increased with increasing aspect ratio, i.e., efficiency was improved by using smaller size beads. Literature data were compared to the broadening theory of Giddings and Mallik (*7*).

4. Discussion

Data (*18*) indicate that mobile phase and stationary phase dispersion processes are independent of each other and contribute additive terms to the resulting plate height in gel chromatography. When the overall plate height is large compared to that due to the mobile phase, it is possible to obtain the stationary phase contribution by subtracting the mobile phase dispersion. Kelley and Billmeyer (*18*) have noted that the plate height term (stationary phase) arising from the permeation into and out of porous glass particles was a linear function of solvent velocity. The linear relationship suggested that broadening due to permeation is controlled by a diffusion mechanism. A diffusion mechanism for retention is consistent with various theories proposed

previously, including those of Yau and Malone (*73*), Casassa (*74, 75*), and Hermans (*76*).

Generally, the plate height equations proposed by Giddings (*7*), Billmeyer (*8, 18*), and by Huber (*10, 11*) show good qualitative agreement with experimental data. Additional results will be required before their relative merits for quantitatively predicting broadening behavior can be assessed accurately. Van Deemter's equation (*1*) does not apply to most broadening results obtained in gel chromatography.

Column efficiency differences related to differences in pore structure between Styragel and Porasil packings suggest that pore geometry is an important factor to be optimized for improved gel filtration systems. A deep interconnected pore structure within the particle should lead to reduced efficiency.

The slowness of achieving radial mixing due to the low molecular diffusivities of macromolecules can be compensated for, in part, by reducing the interparticle distances by using smaller diameter particles while maintaining a relatively high aspect ratio. Smaller particles, however, are more difficult to pack uniformly and agglomeration effects may significantly increase mobile phase dispersion (*70a*). Since molecular diffusivity varies directly with temperature, significant increases cannot be obtained with increased operating temperature.

Little et al. (*7*) have shown that efficient separations are possible at high flow rates. Clearly, for process control applications, future developments will be made for high-speed gel chromatography utilizing small particles, high flow rates, and shorter column lengths, combined with optimization of the pore structure.

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REFERENCES

1. J. J. van Deemter, F. Zuiderweg, and A. Klinkenberg, *Chem. Eng. Sci.*, **5**, 271 (1956).
2. J. C. Giddings, *Anal. Chem.*, **34**, 1186 (1962).
3. J. C. Giddings, *ibid.*, **35**, 439 (1963); **35**, 1338 (1963).
4. J. C. Giddings, *Dynamics of Chromatography, Part 1*, Dekker, New York, 1965.

5. H. F. Walton, in *Chromatography* (E. Heftmann, ed.), Reinhold, New York, 1961, p. 299.
6. S. T. Sie and G. W. A. Rijnders, *Anal. Chim. Acta,* **38,** 3 (1967).
7. J. C. Giddings and K. L. Mallik, *Anal. Chem.,* **38,** 997 (1966).
8. F. W. Billmeyer, Jr., G. W. Johnson, and R. N. Kelley, *J. Chromatogr.,* **34,** 316 (1968).
9. R. N. Kelley and F. W. Billmeyer, Jr., *Anal. Chem.,* **41,** 874 (1969).
10. J. F. K. Huber and J. A. R. J. Hulsman, *Anal. Chim. Acta,* **38,** 305 (1967).
11. J. F. K. Huber, *J. Chromatogr. Sci.,* **7,** 85 (1969).
12. G. W. Johnson, Ph.D. Thesis, Rensselaer Polytechnic Institute, Troy, N.Y., February 1967.
13. J. G. Hendrickson, *J. Polym. Sci., Part A-2,* **6,** 1903 (1968); "Basic Gel Permeation Chromatography Studies VI. Peak Spreading Causes and Evaluation," presented at the 4th International Seminar on Gel Permeation Chromatography, Miami Beach, Florida, May 22–24, 1967.
14. K. H. Altgelt and J. C. Moore, "Gel Permeation Chromatography," in *Polymer Fractionation* (M. Cantow, ed.), Academic, New York, 1967, pp. 123–179.
15. K. H. Altgelt, "Theory and Mechanics of GPC," in *Advances in Chromatography,* Vol. 7 (J. C. Giddings and R. A. Keller, eds.), Dekker, New York, 1968.
16. H. Determan, *Gel Chromatography: Gel Filtration, Gel Permeation, Molecular Sieves,* Springer, New York, 1968.
17. F. W. Billmeyer, Jr., and R. N. Kelley, *J. Chromatogr.,* **34,** 322 (1968).
18. R. N. Kelley and F. W. Billmeyer, Jr., *Anal. Chem.,* **42,** 399 (1970).
19. R. Rhoades, Ph.D. Thesis, Rensselaer Polytechnic Institute, Troy, N.Y., 1963.
20. C. E. Schwartz and J. M. Smith, *Ind. Eng. Chem.,* **45,** 1209 (1953).
21. M. Morales, C. W. Spinn, and J. M. Smith, *ibid.,* **43,** 225 (1951).
22. E. J. Cairns and J. M. Prausnitz, *ibid.,* **51,** 1441 (1959).
23. G. I. Taylor, *Proc. Roy. Soc.,* **A219,** 186 (1953); **A223,** 446 (1954); **A225,** 473 (1954).
24. R. Aris, *ibid.,* **A235,** 67 (1956).
25. T. K. Perkins and O. C. Johnston, *Soc. Petrol. Eng. J.,* **3,** 70 (1963).
26. V. P. Dorweiler and R. W. Fahien, *Amer. Inst. Chem. Eng. J.,* **5,** 139 (1959).
27. J. H. Knox and J. F. Parcher, *Anal. Chem.,* **41,** 1599 (1969).
28. F. W. Grane and G. H. F. Gardner, *J. Chem. Eng. Data,* **6,** 283 (1961).
29. R. J. Blackwell, *Soc. Petrol. Engrs. J.,* **2,** 1 (1962).
30. K. B. Bischoff, *Ind. Eng. Chem.,* **58,** 18 (1966).
31. O. Levenspiel and K. B. Bischoff, *Adv. Chem. Eng.,* **4,** 95 (1963).
32. S. F. Miller and C. J. King, *University of California Radiation Laboratory Report 11951,* May 1965.
33. A. Hennico, G. Jacques, and T. Vermeulen, *University of California Radiation Laboratory Report 10696,* March 18, 1963.
34. D. S. Horne, J. H. Knox, and L. McLaren, *Separ. Sci.,* **1**(5), 531 (1966).
35. M. C. Hawley, Ph.D. Thesis, Michigan State University, East Lansing, Mich., 1964.
36. H. W. Osterhoudt and L. N. Ray, Jr., *J. Polym. Sci., Part A-2,* **5,** 569 (1967).

37. P. E. Pierce, The Glidden Company, Cleveland, Ohio, Private communication, Sept. 1967.
38. M. LePage, R. Beau, and A. J. De Vries, *Polymer Preprints,* 8(2), 1211 (1967); *J. Polym. Sci., Part C,* **21,** 119 (1968).
39. J. B. Carmichael, *Makromol. Chem.,* **122,** 291 (1969).
40. J. B. Carmichael, *J. Chem. Phys.,* **49,** 5161 (1968).
41. J. B. Carmichael, *Macromolecules,* **1,** 526 (1968).
42. J. B. Carmichael, *J. Polym. Sci., Part A-2,* **6,** 572 (1968).
43. H. Vink, *Makromol. Chem.,* **116,** 241 (1968).
44. J. W. Hiby, in *Proceedings of the Symposium on the Interaction Between Fluids and Particles* (P. A. Rottenburg and N. T. Shepherd, eds.), Institution of Chemical Engineers, London, June 20–22, 1962, pp. 312–325.
45. R. A. Bernard and R. H. Wilhelm, *Chem. Eng. Progr.,* **46,** 233 (1950).
46. W. E. Brigham, P. W. Reed, and J. N. Dew, *Soc. Petrol. Eng. J.,* **1,** 1 (1961).
47. R. N. Kelley, Ph.D. Thesis, Rensselaer Polytechnic Institute, Troy, N.Y., June 1969.
48. J. H. Knox, *Anal. Chem.,* **38,** 253 (1966).
49. E. A. Ebach and R. R. White, *Amer. Inst. Chem. Eng. J.,* **4,** 161 (1958).
50. M. LePage and A. J. de Vries, "Evaluation of a New, Versatile Support for G. P. C. Columns: Porous Silica Beads," presented at the 3rd International Seminar on Gel Permeation Chromatography, Geneva, Switzerland, May 1966.
51. A. J. de Vries, M. LePage, R. Beau, and C. L. Guillemin, *Anal. Chem.,* **39,** 935 (1967).
52. C. L. Guillemin, M. LePage, R. Beau, and A. J. de Vries, *ibid.,* **39,** 940 (1967).
53. Pechiney-Saint-Gobain, French Patents 1,473,240; 1,475,929 (1967).
54. W. Haller, *Nature,* **206,** 693 (1965).
55. W. Haller, *J. Chem. Phys.,* **42,** 686 (1965).
56. W. Haller, *J. Chromatogr.,* **32,** 676 (1968).
57. *Price List T,* Bio-Rad Laboratories, Richmond, California, July 1, 1968.
58. E. M. Barrall and J. H. Cain, *J. Polym. Sci., Part C,* **21,** 253 (1967).
59. M. J. R. Cantow and J. F. Johnson, *J. Polym. Sci., Part A-1,* **5,** 2835 (1967).
60. M. J. R. Cantow and J. F. Johnson, *J. Appl. Polym. Sci.,* **11,** 1851 (1967).
60a. A. R. Cooper, J. H. Cain, E. M. Barrall, II, and J. F. Johnson, ACS Div. of Petroleum Chemistry, *Preprints,* **15,** A-95 (1970); Separ. Sci., in press.
61. P. Flodin, *J. Chromatogr.,* **5,** 103 (1961).
62. J. Porath, in *Advances in Protein Chemistry,* Vol. 17 (C. B. Anfinsen, M. L. Anson, K. Bailey, and J. T. Edsall, eds.), Academic, New York, 1962, p. 209.
63. J. Porath and P. Flodin, in *Protides of the Biological Fluids* (H. Peeters, ed.), 10th ed., American Elsevier, New York, 1963, p. 290.
64. V. H. Edwards, Unpublished data.
65. W. B. Smith and A. Kollmansberger, *J. Phys. Chem.,* **69,** 4157 (1965).
66. J. H. Duerksen and A. E. Hamielec, *J. Appl. Polym. Sci.,* **12,** 2225 (1968).
67. W. Heitz and W. Kern, *Angew. Makromol. Chem.,* **1,** 150 (1967).
68. J. Coupek and W. Heitz, *Makromol. Chem.,* **112,** 286 (1968).
69. W. Heitz and J. Coupek, "Column Efficiency in GPC," presented at the 5th International Seminar on Gel Permeation Chromatography, London, England, May 19–22, 1968.

70. J. N. Little, J. L. Waters, K. J. Bombaugh, and W. J. Pauplis, *J. Polym. Sci., Part A-2,* **7,** 1775 (1969).
70a. K. H. Altgelt, Chevron Research Company, Richmond, California, Private communication, Feb. 1970.
71. J. C. Moore and M. C. Arrington, "The Separation Mechanism of Gel Permeation Chromatography Experiments With Porous Glass Column Packing Materials," presented at the 3rd International Seminar on Gel Permeation Chromatography, Geneva, Switzerland, May 1966.
72. F. A. Sliemers, K. A. Boni, D. E. Nemzer, and G. P. Nance, "An Examination of Factors Affecting the Efficiency of GPC Columns Packed With Porous Silica Beads," presented at the 6th International Seminar on Gel Permeation Chromatography, Miami Beach, Florida, October 7–9, 1968.
73. W. W. Yau and C. P. Malone, *J. Polym. Sci., Part B,* **5,** 663 (1967).
74. E. F. Casassa, *ibid.,* **5,** 773 (1967).
75. E. F. Casassa and Y. Tagami, *Macromolecules,* **1,** 526 (1969).
76. J. J. Hermans, *J. Polym. Sci., Part A-2,* **6,** 1217 (1968).

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