Evaluation of Models for Size Exclusion Chromatography of Asymmetric Solutes

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We consider two presentations regarding the size exclusion chromatography of highly asymmetric solutes: one is embodied in the statistical model of Giddings et al.,1 the second is described by the concept of "end-on insertion" proposed by Tanford2 and expanded upon by Meredith and Nathan.3 Our evaluation is based on measurements of Ksec for the rigid rodlike and spherical solutes schizophrenia and ficoll, respectively, and also on measurements of the experimental flow rate dependence of Ksec. The concept of "end-on insertion", as differentiated from standard statistical equilibrium considerations, does not seem to be defensible on fundamental grounds. However, the equilibrium "random pore" treatment of Giddings yields relationships which are incompatible with the current results. The role of the dynamics of solute motion is discussed vis-a-vis hypothetical limitations of equilibrium models.

Introduction

Size-exclusion chromatography (SEC) is a vital technique for the analysis and separation of macromolecules. A variety of models have been proposed regarding the mechanism of SEC (for review see ref 4) without a definite conclusion, although there appears to be relatively little support for nonequilibrium models. The equilibrium models may be divided into two groups. Geometric models place a hard solute in a pore of defined geometry. The partition coefficient, K, is then calculated as the volume available to the center of mass divided by the total volume. This approach has been shown to be successful at predicting the behavior of "hard-sphere solutes".5–6 While it should in principle be applicable to asymmetric solutes as well, no extensive theoretical result has yet been confirmed by experiment. Statistical models predict the partition coefficient as the probability that a randomly positioned solute will not intersect a surface. This analysis is especially useful in the consideration of asymmetric solutes. One of the more frequently cited statistical theories was proposed by Giddings et al.,1 but there has been no published comparison of Giddings' predictions and the experimental partition coefficient, Ksec, as given by

\[ K = \frac{V_c - V_o}{V_i - V_o} \]  

where \( V_c \) is the elution volume, \( V_i \) is the column interstitial volume, and \( V_o \) is the total column volume available to a small solute.

In the Giddings treatment, the magnitude of the partitioning of solute between the mobile and stationary phases of the chromatographic medium is viewed as a consequence of the loss of configurational entropy of the solute when it is confined to a pore relative to that in the bulk solution. When a solute is within a distance equal to its longest linear dimension from the pore wall, there are some configurations which would cause overlap with the wall. These configurations are assumed to be impossible (i.e. the wall is perfectly hard), and the partition coefficient is then defined as the proportion of allowed configurations relative to all configurations. If the solute is at a distance greater than its radius from the wall, all possible configurations are available. Thus, partitioning in SEC is viewed as a surface effect. This model assumes that the system is at equilibrium and the solute is perfectly rigid.

Giddings used two equilibrium statistical models (referred to as "uniform pore network" and "random plane", respectively, and hereafter to models I and II, respectively) to derive SEC relations for a variety of pore and solute geometries. If the solute is spherical and the gel is viewed as a system of uniform cylinders, then one may write

\[ K = \left(1 - \frac{L_o}{d_p}\right)^2 = \left(1 - \frac{sL_o}{d_p}\right)^2 \]  

\[ L_o < d_p \]  

(2a)

\[ K = 0 \]  

\[ L_o \geq d_p \]  

(2b)

where \( L_o \) is the diameter of the spherical solute, \( d_p \) is the pore diameter, and \( s \) is the surface area per unit of free volume, the parameter which describes the stationary phase. For cylindrical pores, \( s = 4/d_p \). Once \( s \) has been determined, it may be used to calculate the partition coefficient for any solute.8 The first equality of eq 2a is also obtained from a purely geometric model of SEC.6

The view of an SEC gel as a system of uniform, cylindrical pores is common but probably simplistic. A somewhat more realistic description results from consideration of the distribution of pore radii.5,9 This may be taken into account by the integral

\[ K = \int_0^\infty K(L_o,s) f(s) \, ds \]  

(3)

where \( f(s) \) is a probability density function describing the distribution of pore sizes. Experimental methods of pore size determination have shown the distribution of Superose 6,9 as well as other supports,10,11 to be approximated by a Gaussian function. However, several factors have prompted us to focus on Giddings' "model II" instead, in which the gel matrix is described in a more general way as a system of randomly intersecting planes.12 This model obviates the need to presuppose a specific pore geometry and also the requirement for information about the distribution of pores, since such distribution is implicit in the model.1

If the mechanism of SEC partitioning is a loss of entropy as a result of surface overlap, then the stationary phase could be modeled by a random array of any surface type. For a set of randomly intersecting planes Giddings has derived the surprisingly simple result

\[ K = \exp\left(-\frac{sX}{2}\right) \]  

(4)

where \( X \) is the mean linear projection length, which is a function

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of solute geometry, and $s$ is the surface area per unit of free volume. Since a distribution of cavities is implicit in the model itself, no further averaging of $s$ is required. For spherical solutes, $X$ is equal to $L_a$. For capsule-shaped solutes (see Figure 1) we have 

$$X = \frac{L_1 + L_0}{2} \tag{5}$$

The quantity $X$ is, by Giddings' treatment, the much sought after "universal calibration" dimension.

Universal calibration has been the focus of much SEC research. The goal is to predict the partition coefficient of any sort of solute from some characteristic dimension. Previously considered dimensions include the Stokes' radius, the viscosity radius, the radius of gyration, the mean linear projection length, and numerous others. The viscosity radius appears to be the universal calibration parameter for globular solutes such as proteins and also for randomly coiled polymers, but significant deviations appear when one considers greatly elongated solutes. Less success has been observed when the Stokes' radius or the radius of gyration is used to predict the elution behavior of different solute types. Elongated solutes have also been shown to elute at volumes different than those of spherical solutes of the same mean linear projection length. Thus, the validity of Giddings' model II, as embodied in eq 6, has already been questioned. Other parameters studied also fail as the universal calibration dimension when highly asymmetric solutes are compared to spherical ones.

The assumed flow rate independence implicit in Giddings' equilibrium model may be contrasted to the flow-dependent consequences of the concept of "end-on insertion" proposed by Tanford and expanded on by Meredith and Nathans. According to this description, the rodlike solute must have the proper orientation to enter the pore. Only a small fraction of these orientations exist at any moment. Thus the number of solutes which enter pores is dependent on the time allowed to do so.

The concept of equilibrium is essential not only to Giddings' theory but also to most of the other models as well. If the system is at equilibrium, then the partition coefficient is independent of transport properties. SEC is thought to be an equilibrium phenomenon for several reasons. First, the partition coefficient is found to be independent of flow rate in all but a few studies. If the solute were adsorbed, the measured partition coefficient would be expected to increase with increasing flow rate. Thus, the dependence of the partition coefficient on the flow rate in some studies may be attributed to electrostatic adsorption phenomena or to structural changes in the chromatographic media. Second, the partition coefficient from static methods is reported to be in agreement with those obtained from SEC. Third, according to calculations based on the diffusion of solute through a network of isotropic spheres by Hermans, a significant deviation from equilibrium should produce excessive spreading and tailing of peaks. The evidence for treating SEC as an equilibrium system is generally good but deserves further discussion.

Yau et al. found the static partition coefficient for polystyrene in chloroform on porous glass to be equal to that obtained from SEC. In contrast, Acker and Brumbaugh, using a direct observation technique, later found excellent agreement between $K_{eq}$ and the partition coefficient obtained from a column saturated with a continuous feed of protein solution. Thus, experiment has shown equilibrium to be well approximated for proteins and randomly coiled polymers under typical conditions, although there are no reports in which static measurements of $K$ have been compared to SEC values obtained over a wide range of flow rates.

Experimental data on the flow rate dependence of highly asymmetric solutes are restricted to the results of Meredith and Nathans and Potschka. Meredith and Nathans found a significant flow rate effect. However, Potschka found no change in the partition coefficient and thus no departure from equilibrium over a wider range of flow rates. In these two studies, no static results were available for comparison. One can only say that the limited experimental evidence appears to support equilibrium conditions for all solutes whether greatly elongated or not.

For a rod with length $L$ greater than the pore diameter, the probability of its entering the pore in time $t$ will be a function of the time for rotation $t_r$ compared to the time for translation over a distance of one rod length $t_L$. In the Giddings treatment, $t_r$ is considered to be essentially infinite. Therefore, the effect of $t_r / t_L$ does not enter into the calculation of $K$. While appreciable flow rates could in principle necessitate the incorporation of such effects, practical SEC is often thought to approximate zero-flow conditions. In contrast, the concept of "end-on insertion" is a dynamic model predicting a dependence on flow rate. If the rotation were fast compared to translation, the time required to attain microscopic equilibrium would be expected to depend on the ratio of rotational to translational relaxation times. This point will be elaborated further.

In the present work, we report on the effects of flow rate for both randomly coiled and rigid asymmetric solutes in order to examine the nature of the equilibrium and to test indirectly the concept of "end-on insertion". We propose to test Giddings' expressions using retention data for schizophyllan, a rodlike macromolecule that displays considerable rigidity within a molecular weight range suited to SEC. It thus corresponds well to the rigid, capsule-shaped solutes of the Giddings theory. The rod length for schizophyllan can be calculated from the relation 

$$L = M / M_o \tag{6}$$

where $M_o$ is 219 Å⁻¹. Since the contour length is $L_1 + L_0$, it is only necessary to divide by 2 to obtain the mean linear projection length.

To evaluate Giddings' equilibrium theory empirically, it is necessary to have an accurate value for $s$. In the case of a gel modeled as a system of random planes, which will be the focus of our comparison with experimental data, $s$ may be obtained from the slope of $-2 \ln K$ versus $L$, as in eq 4. For spherical solutes, the viscosity radius may be used to calculate $L$ since this quantity accounts for the hydrated volume of the solute. The viscosity radius is given by 

$$R_v = \left( \frac{30 [\eta \eta] M}{\pi N_A} \right)^{1/3} \tag{7}$$

where $N_A$ is Avogadro's number and the units of $R_v$ and $[\eta]$ are cm and cm²/g, respectively.

Ficoll was chosen as the solute for determination of $s$ of Superose 6. This densely branched carbohydrate exhibits properties similar to those of a hard sphere. The relation between the intrinsic viscosity, $[\eta]$, and molecular weight, $M$, for ficoll (in pure water at 20 °C) is

$$[\eta] = 3.0 M^{0.75}$$
It is clear that ficoll is not a solid sphere, for which the exponent of \( M \) (the Mark-Houwink parameter) would be zero, but it is far more compact than flexible chain molecules for which \( a \) is typically 0.6–0.7.

**Experimental Section**

**Materials.** Ficoll fractions characterized by light scattering and SEC were a gift from Dr. K. Granath (Pharmacia, Uppsala). The viscosity-average characteristics were determined, as will be discussed later. A broad fraction of schizophyllan (\( M_a = 4.2 \times 10^9 \)) obtained from Dr. T. Yanaki (Taito Co., Kobe, Japan) was sonicated to prepare lower molecular weight fractions as discussed in ref 11. Fractions obtained from Dr. A. Teramoto (Osaka University, Osaka, Japan) were used without further purification. Unpurified DNA Type III (D-1626) from salmon testes was purchased from Sigma Chemical Co. Narrow molecular weight distribution pullulan standards (P-82, Lot. No. 20900) were purchased from SDK Showa Denko, New York.

**Methods.** **Chromatography.** Most chromatography was done on prepacked 1.00 cm i.d. \( \times \) 30 cm Superose 6 columns (Pharmacia, Piscataway NJ). The characteristics of the three columns used for ficoll, schizophyllan, and pullulan, respectively, are shown in Table 1 along with the characteristics of a prepacked TSK SW 4000 (Toyo Soda USA, New York, NY) column employed solely for flow rate studies. Samples were delivered through Milton Roy (Hialeah, FL) Minipumps using Rhodyne Model 7010 injectors (Cotati, CA) with 100 \( \mu \)L loops. The samples were detected with Waters R-401 (Framingham, MA) differential refractometers. All of the values for \( K_{sec} \) except those in flow rate studies were reproducible to within 0.1%.

\( K_{sec} \) of ficoll was measured in 0.1 M phosphate buffer (pH = 7.3) at a flow rate of 0.55 ml/min. Chromatography data for schizophyllan were obtained in 0.38 M, pH 5.5 phosphate buffer (after initial dissolution in 0.01 M NaOH + 0.2 M NaCl, to avoid aggregation), at a flow rate of 0.35 ml/min (from ref 15). We have subsequently shown that such relatively modest variations in pH and ionic strength have a negligible effect on the retention of nonionic polysaccharides on Superose.

Flow rate studies were performed in 0.38 M phosphate buffer (pH = 5.5), except for schizophyllan, which was first dissolved in 0.01 M NaOH + 0.2 M NaCl for the reasons given above, prior to adjustment of pH and ionic strength to match those of the column buffer. The reported retention volumes are the averages of three to four trials.

**Light Scattering.** The translational diffusion coefficient for all schizophyllan fractions except fraction I were measured with a Brookhaven (Holtsville, NY) 72 channel BI-2030 AT digital correlator using a Jodon (Ann Arbor, MI) 15 mW He–Ne laser. The autocorrelation function was analyzed by the method of cumulants. The diffusion coefficient, \( D_e \), of fraction I was extrapolated from the molecular weight dependence of \( D_e \) for the other fractions.

**Results and Discussion**

**Characterization of Solute.** The samples of both ficoll and schizophyllan are polydisperse, requiring a correction so that all measured parameters correspond to the same moment of the distribution. Dubin and Principi found it easiest to correct to the viscosity-average for schizophyllan. A "primary calibration curve" was constructed via an iterative procedure using chromatograms of the schizophyllan fractions along with absolute values for \( M_a \) from ref 14. The chromatographic viscosity-average molecular weight was then calculated from a Mark-Houwink exponent, \( a \), of 1.66 and the relation

\[
[v] = 0.20 M^{0.35} \text{ cm}^3/\text{g} 
\]  

(where \( v \) is the viscosity, \( M \) is the molecular weight, and \( [v] \) is the intrinsic viscosity) and the relation

\[
Dt = (\frac{h_i M_a}{\sum h_i M_a})^{1/2} 
\]  

where \( h_i \) is the height of the \( i \)-th peak division and the initial evaluations of \( M_a \) were based on a calibration curve obtained with pullulan standards. These viscosity-average molecular weights were then used to obtain the corresponding viscosity-average elution volume and partition coefficient. The viscosity-average molecular weights determined by this method were found to agree with those obtained directly by the intrinsic viscosity and the Mark-Houwink relation to within 3%. The viscosity-average data for schizophyllan, along with the contour length calculated by eq 6 and the mean linear projection length, appear in Table 2.

**Flow Rate Dependence.** As shown in Figure 2, no flow effect is seen for schizophyllan or pullulan on Superose 6 in the range 0.2–1.0 ml/min, or on TSK SW 4000 in the range of 1.0–6.0 ml/min. Our results are supported by those of Potschka who found no effects of flow rate on TSK 5000 PW or Zorbax 250 for the rodlike protein tropomyosin in the range 0.02–0.5 ml/min, and who also obtained similar results for the semiflexible rodlike protein \( \alpha \)-actinin on Superose 6. In contrast, Meredith and Nathans found a strong flow rate dependence for the rodlike protein collagen on Sephadex G-50 in the flow rate range 0.04–0.5 ml/min. Several comments may be made in regard to the difference between Potschka's results and ours and those reported by Meredith and Nathans. First, large flow rates effects were not observed consistently in the latter work. For example, fibringen on TSK SW 3000 displayed a decrease in retention volume of

**TABLE 1: Characteristics of Chromatography Columns**

<table>
<thead>
<tr>
<th>column</th>
<th>( V_e )</th>
<th>( V_r )</th>
<th>( n_e )</th>
<th>( \text{efficiency}^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superose I</td>
<td>2600</td>
<td>6.90 &amp;superscript;b</td>
<td>20.44</td>
<td></td>
</tr>
<tr>
<td>Superose II</td>
<td>4100</td>
<td>7.84 &amp;superscript;c</td>
<td>22.42</td>
<td></td>
</tr>
<tr>
<td>Superose III</td>
<td>8300</td>
<td>7.38 &amp;superscript;d</td>
<td>22.05</td>
<td></td>
</tr>
<tr>
<td>TSK SW 4000</td>
<td>5200</td>
<td>5.61 &amp;superscript;d</td>
<td>12.45</td>
<td></td>
</tr>
</tbody>
</table>

\( a \) Theoretical plates; measured with acetone except Superose II measured with D_2O. &superscript;b Measured with polyethylene oxide (10^6M). &superscript;c Measured with blue dextran. &superscript;d Measured with DNA.

**TABLE 2: Viscosity-Averaged Characteristics of Schizophyllan**

<table>
<thead>
<tr>
<th>fraction</th>
<th>( 10^{-4} M_a )</th>
<th>( \sigma, \text{ cm}^3/\text{g}^a )</th>
<th>( L, \text{ Å}^2 )</th>
<th>( V_r, \text{ mL}^a )</th>
<th>( K_v, \text{ mL} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>26.8</td>
<td>174</td>
<td>1220</td>
<td>9.94</td>
<td>0.144</td>
</tr>
<tr>
<td>238</td>
<td>23.4</td>
<td>138</td>
<td>1070</td>
<td>10.18</td>
<td>0.160</td>
</tr>
<tr>
<td>150</td>
<td>14.5</td>
<td>64.0</td>
<td>662</td>
<td>11.52</td>
<td>0.252</td>
</tr>
<tr>
<td>I</td>
<td>11.2</td>
<td>41.4</td>
<td>511</td>
<td>12.12</td>
<td>0.294</td>
</tr>
<tr>
<td>5.49</td>
<td>12.6</td>
<td>251</td>
<td>14.13</td>
<td>0.431</td>
<td></td>
</tr>
</tbody>
</table>

\( a \) Obtained from ref 15. &superscript;b From column 2, via eq 6. &superscript;c From primary calibration curve (see text for explanation).

**TABLE 3: Viscosity-Averaged Characteristics of Ficoll**

<table>
<thead>
<tr>
<th>fraction</th>
<th>( 10^{-4} M_a )</th>
<th>( \sigma, \text{ cm}^3/\text{g}^a )</th>
<th>( R, \text{ Å}^2 )</th>
<th>( V_r, \text{ mL}^a )</th>
<th>( K_v, \text{ mL} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1800-9</td>
<td>70.2</td>
<td>22.2</td>
<td>135</td>
<td>9.31</td>
<td>0.178</td>
</tr>
<tr>
<td>1800-12</td>
<td>48.2</td>
<td>19.5</td>
<td>114</td>
<td>10.20</td>
<td>0.244</td>
</tr>
<tr>
<td>1800-15</td>
<td>31.2</td>
<td>16.7</td>
<td>93.9</td>
<td>11.34</td>
<td>0.328</td>
</tr>
<tr>
<td>1800-20</td>
<td>12.9</td>
<td>12.3</td>
<td>63.2</td>
<td>13.57</td>
<td>0.493</td>
</tr>
<tr>
<td>2500-3</td>
<td>6.84</td>
<td>9.85</td>
<td>47.5</td>
<td>14.86</td>
<td>0.588</td>
</tr>
<tr>
<td>2500-11</td>
<td>2.17</td>
<td>6.58</td>
<td>26.3</td>
<td>16.59</td>
<td>0.716</td>
</tr>
</tbody>
</table>

\( a \) From from primary calibration curve (see text for explanation). &superscript;b From data in column 2, via eq 8. &superscript;c From data in columns 2 and 3, via eq 7.

\[
M_a = \left( \sum h_i M_a^a \right)^{1/a} \sum h_i 
\]  

where \( h_i \) is the height of the \( i \)-th peak division and the initial evaluations of \( M_a \) were based on a calibration curve obtained with pullulan standards. These viscosity-average molecular weights were then used to obtain the corresponding viscosity-average elution volume and partition coefficient. The viscosity-average molecular weights determined by this method were found to agree with those obtained directly by the intrinsic viscosity and the Mark-Houwink relation to within 3%. The viscosity-average data for schizophyllan, along with the contour length calculated by eq 6 and the mean linear projection length, appear in Table 2.

The viscosity-average characteristics of ficoll were obtained in a similar manner. The molecular weights were known by light scattering, allowing the primary calibration curve to be constructed in a more direct manner. The intrinsic viscosity was not measured but was calculated using the Mark-Houwink expression. The viscosity-average characteristics of ficoll appear in Table 3.
only 3–4% when the flow rate was increased from 0.1 to 1.0 mL/min. Second, at the pH employed (7.4) collagen may bear some charge opposite to the weakly anionic charge on Sephadex. The apparently high permeation at low flow rate may in fact be late retention due to adsorptive interactions. Thus collagen, at low flow rates, elutes from Sephadex G-50 at a retention volume expected for proteins with \( M < 5 \times 10^6 \).

**Evaluation of Giddings' Theory.** We now consider Giddings' random plane model (model II) which describes the partition coefficient for any sort of solute in a network of randomly intersecting planes by eq 6. As noted earlier, the value of \( s \) may be determined by a plot of \(-2 \ln K\) versus \(2R_s\), for ficoll, assuming the theory is valid. For Superose 6, this value is \(1.3 \times 10^{-2} \text{Å}^{-1}\) with a correlation coefficient of 0.997. The partition coefficient may then be plotted as a function of the mean linear projection length according to eq 6, along with the experimental data for schizophyllan, as shown in Figure 3. It is clear that the random plane model greatly underestimates \( K \) for rodlike solutes.

It is of interest to note that the relationship between the calibration curves for ficoll and schizophyllan is precisely expressed by the following equation:

\[
R_{\text{eff}}(\text{Å}) = \left( \frac{2}{3} \pi \right)^{1/2} \sqrt{L}
\]

where \( R_{\text{eff}} \) is the radius of a hypothetical ficoll sample of equal \( K_{\text{sec}} \), i.e. the radius of the co-eluting sphere (Table 4). At present the significance of this remarkably simple relation is not clear, in part because of the inconsistency in the units. Nevertheless, the agreement with experiment, as shown in Table 4, is remarkable.

### Table 4: Comparison of Experimental to Predicted Partition Coefficients of Schizophyllan Based on Eq 10

<table>
<thead>
<tr>
<th>Fraction</th>
<th>( K_{\text{sec, exptl}} )</th>
<th>( R_{\text{eff, Å}} )</th>
<th>((2\pi/3)L^{1/2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>0.144</td>
<td>154</td>
<td>153</td>
</tr>
<tr>
<td>238</td>
<td>0.160</td>
<td>146</td>
<td>143</td>
</tr>
<tr>
<td>150</td>
<td>0.252</td>
<td>111</td>
<td>113</td>
</tr>
<tr>
<td>I</td>
<td>0.294</td>
<td>99.6</td>
<td>99.2</td>
</tr>
<tr>
<td>II</td>
<td>0.431</td>
<td>70.5</td>
<td>69.5</td>
</tr>
</tbody>
</table>

* Apparent radius based on ficoll calibration curve.

In the Giddings equilibrium model, the center of mass of the solute occupies all available volume with equal probability. However, if solute rotation were fast with respect to translation, and if the flow time past a pore were short, we might consider a nonequilibrium situation, the result of which would be that \( K \) would depend only on the longest linear dimension of the solute (i.e. the major axis length). Indeed, we previously found \( K \) values for schizophyllan fractions of length \( L \) that approached those of pullulan and proteins with \( R_s = L/2 \) in the limit of small \( L \). However, the fact that we see no flow rate dependence in the present work suggests that we are not in the flow rate regime where such hypothetical departures from equilibrium might occur. Therefore, deviations between the results of experiment and the Giddings treatment are more reasonably attributed to the particular physical description of pore space in model II than to nonequilibrium effects.

**Estimation of Relative Solute Rotation.** Does a rodlike solute in dilute solution during SEC undergo rotational motion more rapidly than translational motion? If so, what bearing does this have on its permeation into the pore? In order to assess the potential role of such dynamic effects, we first consider the role of rotational diffusion. Derivation of the standard translational diffusion expression assumes that rotation is fast compared to the time or distance of measurement. Thus, the translational diffusion must be measured over a distance or length such that all rotational orientations are sampled. This distance, \( \Delta x \), may be calculated by equating the translational diffusion time with the time for rotation \( \tau_r \):

\[
\Delta x^2 = 2D_t \tau_r \approx 2D_i/D_r = (2kT/f_j)\tau_r
\]

\( D_t \) and \( D_i \) are respectively translational and rotational diffusion constants, and \( f_j \) is the translational friction coefficient. For asymmetric bodies, the translational and rotational diffusion constants are complicated functions of shape. Solutions for rotational ellipsoids are well-known, and approximations for rods are available. From ref 28, one obtains, for axial ratios \( L_1/L_0 > 5 \):

\[
\Delta x \approx L_1/3 \approx 2X/3
\]

We are left to conclude that, on the average, a rod has sampled all of its rotational orientations after a translational path one-third its length has been traversed.

The entry of a rod-shaped molecule of length \( L \) into a cylindrical pore of diameter \( d_p \) where \( L > d_p \) requires first orienting the molecule with the pore axis, followed by translation without significant rotation until entry is established. Assessing the likelihood of this involves a comparison of the distance \( \Delta x \) to some characteristic length for the chromatographic process, namely the pore dimension. For most solute molecules, for which \( d_p \gg \Delta x \), the path into the stagnant zone (pore) may be considered to be rotationally averaged. The case for schizophyllan is more complex, since the contour lengths of the larger samples studied here exceed the pore diameter. For such samples, the result shown above, that \( \Delta x \approx L_1/3 \), makes the entry for a single encounter unlikely, although, at "equilibrium"—given sufficient number of encounters—entry will occur. The situation is complicated by the addition of flow, the effect of which depends on the orientation

![Figure 2. Effect of flow rate on retention of schizophyllan II (O), pullulan 800 (\( \Delta \)), and pullulan 400 (\( \square \)) on a Superose 6 column.](image1.png)

![Figure 3. Experimental results for schizophyllan on Superose 6 (O), compared to values calculated from Giddings' random plane model for capsule-shaped solutes.](image2.png)
of the pore opening with respect to local flow. A distribution of pore orientations could then contribute to symmetric band-broadening with no change in $K_{sec}$. One could also state that the presence of flow imposes another time scale, in addition to the times of rotation ($\tau_1$) and of translation into a pore ($\tau_{inw}$): the time during which a molecule flows past a pore ($\tau_{flow}$). Although the heterogeneity of pore sizes, geometries, and orientations makes it evident that this is a complex average, there is nevertheless an inverse relationship between the flow rate and the number of orientations sampled for a given molecule during its proximity to a given pore. The fact that large flow rates induce band-broadening with no change in $K_{sec}$ makes it evident that this is a complex average, there is nevertheless an inverse relationship between the flow rate and the number of orientations sampled for a given molecule during its proximity to a given pore. The fact that large flow rates induce band-broadening in SEC may indicate that this last time could be of the same order of magnitude as the time for pore entry.

At sufficiently high flow rate one would expect asymmetric band-broadening and alteration of $K_{sec}$. However, the local flow adjacent to a pore opening may be significantly less than the bulk flow due to microscopic hydrodynamics, so that the absence of measurable flow rate effects may be a reflection of the difficulty of achieving significant longitudinal transport in the vicinity of the pore entrance at experimentally attainable bulk flow rates. Molecular simulation of the flow-dependence of $K$ would have to incorporate the local hydrodynamics, Langevin forces on the solute, the orientation distribution of the pores, and the time dependence of flow within the pores and would therefore be a formidable task. Experimentally, it may be that the effect of $\tau_{i}/\tau_{flow}$ could be better observed by modification of mobile-phase viscosity, instead of macroscopic flow rate.

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References and Notes

(2) Nozaki, Y.; Schechter, N. M.; Reynolds, J. A.; Tanford, C. Biochemistry 1976, 15, 3884.
(8) Reference 1 provides equations for the partition coefficient of rod-shaped solutes in uniform cylindrical pores. One of the reviewers pointed out a typographical error in ref. 1: in eq 18a the first "$1 - \beta^2$" term should be "$1 + \beta^2$".
(12) Initial attempts to use model 1 as given by Giddings led to values of $K > 1$, indicating that the theoretical expression as given in ref 1 is incorrect (see ref 8).
(30) Koenig, S. H. Biopolymers 1975, 14, 2421.