An Equilibrium Theory for Exclusion Chromatography of Branched and Linear Polymer Chains[1]

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ABSTRACT: The equilibrium partitioning of random-flight polymer chains between a dilute macroscopic solution phase and liquid within cavities of macromolecular size is obtained from the mathematical formalism for Brownian motion of particles within a bounded region. The derivation applies to linear chains and branched "star" molecules in a Θ solvent and to cavities of three shapes—a sphere, an infinite cylinder, and the space between two parallel infinite planes. Chromatographic theory suggests that conditions under which the equilibrium distribution coefficient for a polymer solute determines its elution volume should be easily attained in permeation columns packed with material containing rigid pores. It is found that elution volumes calculated solely from equilibrium thermodynamics agree closely with predictions based on the assumption that the elution volume for any flexible chain species, linear or branched, depends on the effective hydrodynamic volume of the polymer molecule.

In a recent communication,[2] we outlined the derivation of equations to predict how flexible linear polymer chains dissolved in a Θ solvent are distributed at equilibrium between a very dilute macroscopic solution phase and the liquid in cavities of macromolecular size. Three simple shapes were considered for the voids—the sphere, the infinite cylinder, and the volume bounded by two parallel infinite planes (*i.e.*, a slab). The voids were taken to be completely permeable to solvent and their walls were regarded simply as rigid boundaries exerting no specific forces on either polymer or solvent. Our motive was to formulate a theory valid at the equilibrium limit for the separation effect in what is variously described as gel permeation,[3,4] gel filtration,[5] or exclusion[6] chromatography. (The first term is perhaps the most popular today; but the last seems most appropriate here, in view of the physical picture conveyed by our theoretical models.)

In this paper we retain the simplifying assumptions made in our earlier account and limit consideration to a polymer in a Θ solvent in contact with a porous rigid matrix containing uniform voids of simple geometrical form. The geometry of the voids, chosen for reasons of mathematical simplicity, can hardly represent accurately the pores in any real chromatographic medium; but since we shall be more interested in the polymer than in the pore matrix, this should not be a serious deficiency. That is, we expect to find that the comparative behavior of two different polymers in a particular porous medium can be characterized in a way that is rather insensitive to the geometry of the pores.

Our chief aim in this paper is to effect a generalization of the earlier treatment to branched chains. Comparisons between linear and branched chains are of particular importance since various workers have suggested, on the basis of experimental data or intuitive assumptions, one or another parameter of a polymer chain as providing a "universal" criterion[7-15] governing elution from any given chromatographic column. Introduction of branching as a variable in chain structure permits varying the molecular dimensions without changing the mass of the molecule; and thus, studies of linear and branched chains may well provide the most stringent tests of such proposals.

In what follows we first calculate the distribution of star-shaped branched polymers (of which linear chains are a pathological case) between the "outside" solution and the space inside voids; and then we examine the bearing of the results on exclusion chromatography.

Derivation of the Distribution Coefficient. 1. Application of the Diffusion Equation. To represent a branched polymer molecule we assume the "regular star" model,[16,17] f identical linear chains all joined together at one end. Accordingly, we can imagine a star conformation to be generated by beginning f random flights at a single point and allowing each to proceed for n steps. The mathematical formalism of Brownian motion supplies the technique for solution of what is essentially a question of enumerating chain configurations. Therefore, as in the earlier work,[2] we seek appropriate solutions of the diffusion equation (eq 1),

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(1) This work was presented at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1-5, 1968.

(2) E. F. Casassa, *J. Polym. Sci., Part B*, **5**, 773 (1967).

(3) J. C. Moore, *ibid., Part A*, **2**, 835 (1964).

(4) J. C. Moore and J. G. Hendrickson, *ibid., Part C*, **8**, 233 (1965).

(5) J. Porath and P. Flodin, *Nature*, **183**, 1657 (1959).

(6) K. O. Pedersen, *Arch. Biochem. Biophys. Suppl.*, **1**, 157 (1962).

(7) L. E. Maley, *J. Polym. Sci., Part C*, **8**, 253 (1965).

(8) G. Meyerhoff, *Makromol. Chem.*, **89**, 282 (1965); *Ber. Bunsenges. Phys. Chem.*, **69**, 866 (1965).

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(12) Z. Grubisic, P. Rempp, and H. Benoit, *J. Polym. Sci., Part B*, **5**, 753 (1967).

(13) L. Wild and R. Guliana, *ibid., Part A-2*, **5**, 1087 (1967).

(14) J. B. Carmichael, *ibid., Part A-2*, **6**, 517 (1968).

(15) K. A. Boni, F. A. Sliemers, and P. B. Stickney, *ibid., Part A-2*, **6**, 1579 (1968).

(16) B. H. Zimm and W. H. Stockmayer, *J. Chem. Phys.*, **17**, 1301 (1949).

(17) T. A. Orofino, *Polymer*, **2**, 295, 305 (1961).

$$\frac{\partial P_n(\mathbf{r})}{\partial n} = \frac{b^2}{6}\nabla^2 P_n(\mathbf{r}) \quad (1)$$

where P_n ($\mathbf{r}$)d$\mathbf{r}$ is the probability for finding the nth step of a random flight within a differential element of space d$\mathbf{r}$ at a point labeled by the vector $\mathbf{r}$, and b^2 is the mean square step length (or statistical segment length in a polymer chain) assumed to be normally distributed. The differentiation with respect to n implies that n is to be regarded as very large. The exact analogy with the differential equation for heat conduction—with $P_n(\mathbf{r})$ corresponding to the temperature at the point $\mathbf{r}$, and n to the time—requires no justification here; it is, however, important to the present discussion because many of the calculations we encounter appear in connection with simple problems in heat flow that have long ago been solved. A comprehensive reference is the well-known treatise by Carslaw and Jaeger[18] (hereafter identified by CJ).

With the aid of the diffusion equation, we determine the extent to which the conformational freedom of a polymer is decreased when it is transferred from the bulk solution phase to a void. For example, we can imagine that a linear chain has one end at a point $\mathbf{r}'$ inside a void and inquire as to the probability density $P_n(\mathbf{r}|\mathbf{r}',S)$ for finding the nth step at another point $\mathbf{r}$ inside the void, and all earlier steps inside a mathematical surface S that reproduces the boundary of the void but does not impede the random flight. In other words, $P_n(\mathbf{r}|\mathbf{r}',S)d\mathbf{r}$ is the probability that a random flight of n steps beginning at $\mathbf{r}'$ ends within d$\mathbf{r}$ without intersecting S. As Hollingsworth,[19,20] then Weidmann, *et al.*,[21] and, more recently, DiMarzio[22] have pointed out, this probability is obtained by solving the diffusion equation with an "absorbing" boundary at S; that is, all $P_n(\mathbf{r})$ vanish for $\mathbf{r}$ on S. Hence, all chain conformations that touch the boundary are obliterated. Averaging over all $\mathbf{r}'$ and $\mathbf{r}$ within S then gives the fraction of all chain conformations originating within the volume bounded by S, but otherwise unrestricted, that is still allowed when an impenetrable wall is placed at S. This fraction of allowed chain conformations is the distribution coefficient, the equilibrium ratio of the concentration of polymer in the space inside voids to that in the macroscopic phase outside.

To obtain the distribution coefficient for a branched chain we can first calculate $P_n(\mathbf{r}|\mathbf{r}',S)$ for one of the branches of n linear steps beginning at the branch point placed at $\mathbf{r}'$. Then we integrate over $\mathbf{r}$ within S to obtain the probability, which we shall designate as $P_{r'}$, that the linear chain of n elements beginning at $\mathbf{r}'$ lies entirely within the boundary. For the branched molecule there are f chains of n steps, all beginning at $\mathbf{r}'$. Consequently, the probability that a star with the branch point at $\mathbf{r}'$ has its conformation entirely inside S, is $(P_{r'})^f$. Integration over all $\mathbf{r}'$ inside S then leads to the distribution coefficient K_f for the star molecule. The occurrence of only one branch point in the star model represents a major simplification. Allowing nonuniformity in the length of the arms does not affect the derivation basically, but topologically more complex branched structures will obviously be more difficult to deal with.

2. Solutions for Cavities of Simple Geometry. In fact, the calculation sequence just outlined, though conceptually straightforward, is not usually the easiest procedure; but it does serve for a void of the simplest geometrical shape, a slab bounded by two infinite parallel planes separated by a distance l. Using a rectangular coordinate system with the planes at $z = 0$ and $z = l$, we begin a random flight at a point $0,0,z'$ between the two planes. The probability density for finding an unrestricted chain of n steps entirely inside the slab *and* the nth segment at x,y,z is

$$P(x,y,z|0,0,z';l) = \left(\frac{3}{\pi n b^2 l}\right)\exp\left[-\frac{3(x^2+y^2)}{2nb^2}\right]\sum_{p=1}^{\infty}\exp\left(-\frac{p^2\pi^2 n b^2}{6l^2}\right)\times \sin\left(\frac{p\pi z}{l}\right)\sin\left(\frac{p\pi z'}{l}\right) \quad (2)$$

Integration over x, y, and z gives the probability that a random flight beginning at any point x',y',z' remains inside the slab.

$$P_{z'} = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{z=0}^{l} P(x,y,z|z',l)\,\mathrm{d}x\mathrm{d}y\mathrm{d}z = \frac{4}{\pi}\sum_{p\ odd}\frac{1}{p}\exp\left(-\frac{p^2\pi^2 n b^2}{6l^2}\right)\sin\left(\frac{p\pi z'}{l}\right) \quad (3)$$

To obtain the averaged probability that the linear chain beginning somewhere inside the slab does not intersect the boundary we integrate $P_{z'}$ over $0 \leq z' \leq l$ and divide by l to normalize the result. For the regular-star molecule containing nf segments, the distribution coefficient is

$$(K_f)_{\text{slab}} = \frac{1}{l}\int_0^l (P_{z'})^f\,\mathrm{d}z' = \left(\frac{4}{\pi}\right)^f \underbrace{\sum_p\sum_q\cdots\sum_t}_{odd\ integers}\frac{1}{pq\ldots t}\times \exp\left[-\frac{(p^2+q^2+\ldots+t^2)\pi^2 n b^2}{6l^2}\right]\times \frac{1}{l}\int_0^l \sin\left(\frac{p\pi z'}{l}\right)\sin\left(\frac{q\pi z'}{l}\right)\ldots\sin\left(\frac{t\pi z'}{l}\right)\mathrm{d}z' \quad (4)$$

where the sum is to be understood as f-fold. When f is unity, equation 4 reverts to the power series for a linear chain.[2] The same result is obtained when $f = 2$; then the integral assumes the familiar form of the orthogonality condition, being equal to $l/2$ when the indices are alike and vanishing when they are different. Evaluation of the integrals for $f > 2$ proceeds without difficulty since by use of elementary trigonometric identities the multiple product of sines can be reduced to a sum of terms, each containing a single sine

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(18) H. S. Carslaw and J. C. Jaeger, "Conduction of Heat in Solids," 2nd ed, Oxford University Press, London, 1959; (a) Chapter 10, eq 14.10(12); (b) eq 3.3(10); (c) eq 14.7(7); (d) eq 14.8(5) and 7.6(5); (e) eq 3.3(8); (f) eq 9.3(5); (g) 13.3(3); (h) eq 2.4(10).

(19) C. A. Hollingsworth, *J. Chem. Phys.*, **16,** 544 (1948).

(20) C. A. Hollingsworth, *ibid.*, **17,** 97 (1949).

(21) J. J. Weidmann, H. Kuhn, and W. Kuhn, *J. Chim. Phys.*, **50,** 266 (1953).

(22) E. A. DiMarzio, *J. Chem. Phys.*, **42,** 2101 (1965).

or cosine of a linear combination of angles. For values of f and nb^2/l^2 of interest many terms of the series may be needed for accurate calculation of $(K_f)_{\text{slab}}$. Fortunately, as is indicated in the Appendix, the results can be systematized to facilitate computer calculations for f of any order.

Equation 2 is formally analogous to the expression for the temperature at time t at a point x,y,z in a homogeneous conducting slab due to an instantaneous point source of heat introduced at zero time at $0,0,z'$. The surface of the slab is held at zero temperature for all time; *i.e.*, the slab is immersed in a large bath at zero temperature.[18a] Then, the integration leading to eq 3 corresponds to a calculation of the mean temperature of the slab at time t after the introduction of an instantaneous plane source at z'. For the case $f = 1$, the succeeding calculation of K is analogous to determining the mean temperature at t of a slab initially at uniform temperature, or, equivalently, with instantaneous sources densely and uniformly distributed.[18b] In terms of the random-flight chain, this picture corresponds to distributing the initial steps of many chains uniformly throughout the slab-shaped void and inquiring as to what fraction have not intersected the boundary after each has executed n steps. The idea of a uniform density of chain origins in introduced here because adoption of this point of view simplifies the symmetry for the pore geometries to be discussed next, and in effect reduces each situation to a one-dimensional problem.

Of other models for voids, the sphere is perhaps the most tractable; and it thus has some interest despite the obviously physical difficulty that a void of strictly spherical form provides no way for solute or solvent to enter.

We distribute the starting points for a large number of random flights within a spherical region $0 \leq r < a$ so that the density ρ of points is everywhere uniform. The number of chains beginning in the spherical shell between radii r' and $r' + \mathrm{d}r'$ is then $4\pi(r')^2\rho\mathrm{d}r'$. The number of chain ends (after n steps) lying between r and $r + \mathrm{d}r$ and resulting from chains started in a unit volume at r' is $4\pi r^2\rho P(r|r',a)\mathrm{d}r$ where

$$P(r|r',a) = \frac{1}{2\pi arr'}\sum_{p=1}^{\infty} \sin\left(\frac{p\pi r}{a}\right) \sin\left(\frac{p\pi r'}{a}\right) \exp\left(-\frac{p^2\pi^2nb^2}{6a^2}\right) \tag{5}$$

is the density of chain ends at r resulting from chains generated at unit-density at r'.

The analog to eq 5 in heat conduction is the temperature distribution as a function of time resulting from an instantaneous spherical-shell source of heat introduced at the radius r' in a sphere of isotropic material with the boundary $r = a$ at zero temperature.[18c]

Integrations over r and r' are elementary and lead to the distribution coefficient for a linear chain;[2] but as we did in the case of the slab, to derive the general case we first integrate over r to obtain eq 6, which is the fraction of unrestricted linear chains generated at r' that remains inside the sphere for all steps or, in other words, the probability that a chain generated at $r' < a$ will not intersect the boundary at $r = a$. To generalize this result for branched chains beginning anywhere in the sphere, we have of course to consider f linear chains beginning at r', and average $(P_{r'})^f$ over the entire spherical volume V_a (eq 7), where the index runs over all positive integers.

$$P_{r'} \equiv \frac{1}{\rho}\int_0^a 4\pi r^2\rho P(r|r',a)\mathrm{d}r = \frac{2a}{\pi r'}\sum_{p=1}^{\infty}\frac{(-1)^{p+1}}{p} \times \sin\left(\frac{p\pi r'}{a}\right)\exp\left(-\frac{p^2\pi^2nb^2}{6a^2}\right) \tag{6}$$

$$\begin{aligned}(K_f)_{\text{sphere}} &= \frac{1}{V_a}\int_{V_a}(P_{r'})^f\,\mathrm{d}V \\ &= 3a^{f-3}\left(\frac{2}{\pi}\right)^f\int_0^a\frac{1}{(r')^{f-2}}\left[\sum_{p=1}^{\infty}\frac{(-1)^{p+1}}{p} \times \exp\left(-\frac{p^2\pi^2nb^2}{6a^2}\right)\sin\left(\frac{p\pi r'}{a}\right)\right]^f\mathrm{d}r'\end{aligned} \tag{7}$$

Like $(P_{z'})^f$ in eq 4, the integrand in eq 7 is expanded in the form of a multiple summation, each term containing a product of f sines. Again, simple transformations permit reduction of each product to a sum of terms each containing one sine or cosine of a multiple of $\pi r'/a$. Now, however, because of the presence of the factors $(r')^{f-2}$ in the denominator, the integrals cannot be evaluated in terms of elementary functions. Some manipulation establishes that results can always be written in terms of integral sine functions

$$\mathrm{Si}\,(x) = \int_0^x \frac{\sin u}{u}\,\mathrm{d}u$$

of multiples of π. Values of Si $(m\pi)$, with m a positive integer, have been tabulated.[23] The calculations fall into a pattern similar to that encountered in the case of the slab model. Some details are given in the Appendix.

The last model we investigate here is the circular cylindrical void of indefinite length. It is the most interesting of the three on grounds of physical plausibility, but unfortunately it is the most awkward mathematically. We imagine a cylindrical region $0 \leq r < a$ and within it a cylindrical shell at r'. The probability density for finding chain ends at radius r due to a unit volume element at r' containing a unit density of chain origins is

$$P(r|r',a) = \frac{1}{\pi a^2}\sum_{p=1}^{\infty}\exp\left(-\frac{\alpha_p{}^2nb^2}{6}\right)\frac{J_0(\alpha_p r)J_0(\alpha_p r')}{J_1{}^2(\alpha_p a)} \tag{8}$$

where the α_p are the positive roots of the equation

$$J_0(\alpha a) = 0$$

and $J_0(x)$ and $J_1(x)$ have the conventional meaning of Bessel functions of the first kind of order zero and one. The fraction of chains generated at r' that remains within the cylinder for all n steps is given by eq 9. The

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(23) Y. L. Luke and J. Wimp, *Mathematics of Computation*, 17, 395 (1963). A short table is given by W. Gautschi and W. F. Cahill, in "Handbook of Mathematical Functions," M. Abramowitz and I. A. Stegun, Ed., National Bureau of Standards Applied Mathematics Series, No. 55, 1964.

$$(P_{r'})_{\mathrm{cyl}} = \int_0^a 2\pi r P(r|r',a)\,\mathrm{d}r =$$

$$\frac{2}{a}\sum_{p=1}^{\infty}\frac{1}{\alpha_p}\exp\left(-\frac{\alpha_p{}^2nb^2}{6}\right)\frac{J_0(\alpha_p r')}{J_1(\alpha_p a)} \quad (9)$$

analogous relations for heat conduction in a cylinder are given in CJ.[18d]

Finally, the distribution coefficient for a star molecule in the hollow cylinder is obviously

$$(K_f)_{\mathrm{cyl}} = \frac{1}{\pi a^2}\int_0^a 2\pi r'(P_{r'})^f\,\mathrm{d}r'$$

$$= \frac{1}{a}\left(\frac{2}{a}\right)^{f+1}\int_0^a\left[\sum_{p=1}^{\infty}\frac{1}{\alpha_p}\exp\left(-\frac{\alpha_p{}^2nb^2}{6}\right)\times\frac{J_0(\alpha_p r')}{J_1(\alpha_p a)}\right]^f r'\,\mathrm{d}r' \quad (10)$$

For $f = 1$, we recover the result for a linear chain; and the same expression is obtained for $f = 2$ by invoking the well-known property of Bessel functions

$$\int_0^a J_0(\alpha_p r)J_0(\alpha_q r)r\,\mathrm{d}r = \begin{cases}0 & (p \neq q)\\(a^2/2)J_1{}^2(\alpha_p a) & (p = q)\end{cases}$$

When $f > 2$, expansion of the integrand in eq 10 as a multiple summation leads to integrals of the form

$$\int_0^a J_0(\alpha_p r)J_0(\alpha_q r)\ldots J_0(\alpha_t r)r\,\mathrm{d}r$$

and these defy analytical solution. Apparently, the most that has been accomplished is to transform the integral containing a product of three Bessel functions to one not further reducible with only one Bessel function in the integrand.[24] We have, therefore, obtained K_f for the cylinder by numerical integration of eq 10.

3. Solutions for K_f near Unity. The series solutions of the diffusion equation obtained above—solutions for long times in the heat conduction analogy—converge rapidly when nb^2/a^2, or nb^2/l^2, is large. Convergence is actually reasonable whenever K is much less than unity; hence these exponential forms were used in computing numerical values of K_f for graphical presentation. It is also possible to write solutions convergent near $K = 1$ (the "short time" solutions in heat flow) in terms of error functions. Considering, for example, the slab model, placing the origin at the middle of the slab, and letting $a = l/2$, we can write, instead of eq 3 and 4

$$(K_f)_{\mathrm{slab}} = \frac{1}{a}\int_0^a (P_{z'})^f\,\mathrm{d}z' \quad (11)$$

and

$$P_{z'} = 1 - \sum_{p=0}^{\infty}(-1)^p\left\{\mathrm{erfc}\left[\frac{(2p+1)a - z'}{2(nb^2/6)^{1/2}}\right] + \mathrm{erfc}\left[\frac{(2p+1)a + z'}{2(nb^2/6)^{1/2}}\right]\right\} \quad (12)$$

In eq 12, erfc denotes the error function complement

$$\mathrm{erfc}(x) = 1 - \mathrm{erf}(x) = 1 - \frac{2}{\sqrt{\pi}}\int_0^x e^{-\xi^2}\,\mathrm{d}\xi = \frac{2}{\sqrt{\pi}}\int_x^{\infty} e^{-\xi^2}\,\mathrm{d}\xi$$

The counterpart of eq 12 for heat conduction is given in CJ.[18e] Similar relations can be formulated for the other cavity shapes (*cf.* Appendix below and CJ[18f,g]).

Although we have not needed the error function solutions in numerical computations of K_f, they are useful in determining the limiting dependence of K_f on f and nb^2/a^2 as R/a vanishes—*i.e.*, as permeation of voids is complete and $K_f = 1$. We can expand $(P_{z'})^f$ in eq 11 by the binomial theorem and integrate the resulting series term by term with respect to z'. These operations (shown in detail in the Appendix) lead to a common limiting form for all three models of cavities

$$K_f = 1 - 2\lambda\psi(f)f^{1/2}(nfb^2/6a^2)^{1/2} + \mathrm{O}(nb^2/6a^2) \quad (13)$$

where

$$\lambda = \begin{cases}1 \text{ for the slab}\\2 \text{ for the cylinder}\\3 \text{ for the sphere}\end{cases}$$

and

$$\psi(f) = \left(\frac{f}{\pi}\right)^{1/2}\int_0^{\infty}[\mathrm{erf}\,(t^{1/2})^{f-1}e^{-t}\,\mathrm{d}t \quad (14)$$

In particular, for the linear chain, we have

$$\psi(1) = \psi(2) = \pi^{-1/2}$$

The important property of eq 13 is that the coefficient of $(nb^2/6a^2)^{1/2}$ is a product of two factors, one depending only on the shape of the cavity, and the other only on the number of branches in the permeating species.[25]

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[24] H. E. Fettis, *J. Math. Phys.*, **36**, 88 (1957).

[25] Intuition indicates a simple route to eq 13. A less detailed picture of molecular exclusion than we have elaborated here is inspired by the idea that a cavity might be characterized merely by a total surface S near which the conformational freedom of dissolved polymer chains is restricted and, therefore, the concentration is reduced. If this surface is taken as an infinite plane bounding the region $x > 0$, the probability that a configuration of an *unconfined* linear chain of n steps beginning at a distance x' from the plane does not intersect it is

$$P_{x'} = \mathrm{erf}\left[\frac{x'}{2}\left(\frac{6}{nb^2}\right)^{1/2}\right]$$

(in accord with CJ[18h]). The net decrease in polymer concentration in the region $x > 0$ due to the presence of the boundary is then equivalent to that produced by allowing no chains to begin within a layer of solution of thickness

$$x_c = \int_0^{\infty}(1 - P_{x'})\,\mathrm{d}x' = 2(nb^2/6\pi)^{1/2}$$

adjacent to the boundary and placing a uniform concentration of chain origins throughout $x > x_c$. The generalization to star molecules is obvious from the calculations of K_f for the cavity models. The effective distribution coefficient for any cavity of volume V is then given by the expression $K = (V - Sx_c)/V$, which corresponds to eq 13. This equivalence has an obvious explanation in the fact that the limit $K = 1$ corresponds to the situation of a polymer chain in an extremely large cavity, which, whatever its shape, presents to the polymer a barrier deviating only infinitesimally from a plane surface. In the appendix we show that the effect of the shape of the cavity on x_c does indeed only appear in terms of order higher than $(nb^2/a^2)^{1/2}$.

Further, the shape-dependent factors are in the same proportion as the surface-to-volume ratios of the cavities.

Dependence of the Distribution Coefficient on Molecular Weight and Chain Branching. In Figure 1, plots of K_f are shown for the three pore shapes and several values of f. The abscissa is the dimensionless ratio

$$(Nb^2/6a^2)^{1/2} = R/ag^{1/2}$$

where $N = nf$ is the total number of chain segments in the molecule and a is, as before, the radius for the cylinder and for the sphere, and half the separation of the faces for the slab. The equality follows from the random-flight mean-square radius of a linear polymer chain

$$(R^2)_{\text{lin}} = Nb^2/6$$

and the usual definition of g

$$g \equiv R^2/(R^2)_{\text{lin}}$$

as the ratio of the unperturbed mean-square radius of the chain in question to that of a linear chain of the same mass. For regular stars[16] we have

$$g = (3f - 2)/f^2$$

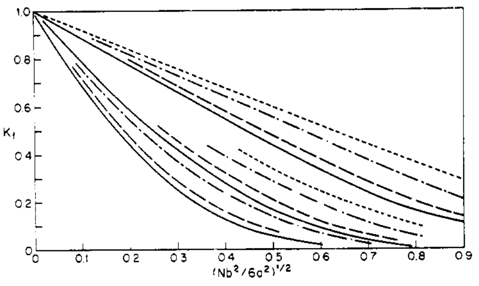

Figure 1. Distribution coefficients K_f: reading from bottom to top; $(K_1)_{\text{sphere}}$, $(K_4)_{\text{sphere}}$, $(K_8)_{\text{sphere}}$, $(K_1)_{\text{cyl}}$, $(K_4)_{\text{cyl}}$, $(K_8)_{\text{cyl}}$, $(K_{12})_{\text{cyl}}$, $(K_1)_{\text{slab}}$, $(K_4)_{\text{slab}}$, $(K_8)_{\text{slab}}$, $(K_{12})_{\text{slab}}$. The curve for $(K_{12})_{\text{sphere}}$ (not shown) lies close to that for $(K_1)_{\text{cyl}}$.

Qualitatively, the results shown in Figure 1 are in accord with expectations. We see that for a given polymer molecule and a given value of a, the partition coefficient, and thus the degree of permeation, is greatest for the slab and least for the sphere–as is to be expected inasmuch as the slab and the sphere boundaries produce, respectively, the least and the greatest decrease in the number of available conformations for a given chain. Then, considering a given cavity we see that K_f decreases as N is increased at fixed f, and thus as the mean-square radius is increased. Conversely, holding N fixed as f is increased causes K_f for a given cavity to increase; *i.e.*, K_f increases as a molecule of given mass is made more compact by increasing the amount of branching.

Since K_f is not uniquely dependent on the number of segments in a chain, irrespective of branching, for a cavity of given size and shape, it is natural to inquire whether the mean-square molecular radius can serve to correlate distribution coefficients for linear and branched chains. However, plots of K_f *vs.* R/a (not given) show that the curves for K_f for a given pore model are even farther from coincidence than the comparable curves in Figure 1. The order of the curves is reversed, with K_f at fixed R/a decreasing with increasing f; and thus the change in the abscissa by a factor of $g^{1/2}$ overcompensates for the actual dependence on molecular radius. In Figure 2 we show an intermediate choice with K_f plotted against $(Nb^2/6a^2)^{1/2}g^{1/6}$ for the sphere and slab models. It is apparent that reasonable superposition of the plots is achieved. Agreement is close when f is small (the curves for $1 \leq f \leq 4$ are practically indistinguishable), but powers of g slightly larger than one-sixth are necessary at large f to force a fit with the curve for $f = 1$.

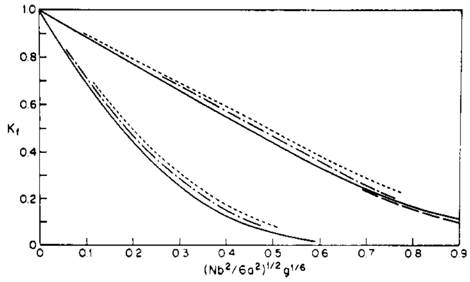

Figure 2. Dependence of K_f on $(R/a)g^{-1/3}$: reading from bottom to top; $(K_1)_{\text{sphere}}$, $(K_8)_{\text{sphere}}$, $(K_{12})_{\text{sphere}}$, $(K_4)_{\text{slab}}$, $(K_1)_{\text{slab}}$, $(K_8)_{\text{slab}}$, $(K_{12})_{\text{slab}}$. The curve for $(K_4)_{\text{sphere}}$ nearly coincides with that for $(K_1)_{\text{sphere}}$. Similarly $(K_4)_{\text{slab}}$ is indistinguishable from $(K_1)_{\text{slab}}$ except when K is small.

Introduction of the factor $g^{1/6}$ is proposed in this context merely on the basis of curve fitting; but we shall comment on this matter again in connection with correlation of experimental data. This adjustment cannot apply when K_f is very small. In fact, the *unadjusted* curves shown in Figure 1 superpose correctly for small K in the sense that the ratio of abscissas corresponding to equal values of K_1 and K_f, for the same cavity shape, approach unity at the limit; and this agreement is destroyed by any scaling of the abscissas to force a fit elsewhere.

At the limit of high permeation the dependence of K_f on f is determined by the function ψ in eq 13. Results obtained by numerical integration are listed in Table I, together with the ratio g and values of the exponent ν required by the relation

$$\pi^{1/2}\psi = g^{\nu} \qquad (15)$$

The slope of K_f *vs.* $(Nb^2/6a^2)^{1/2}g^{\nu}$ at $K_f = 1$ coincides with that for an unbranched chain. These powers of g may be compared with the factor $g^{1/6}$ chosen as an arbitrary compromise, without regard to the initial slopes, for plotting Figure 2. The similarity of the initial slopes to the factor g^{ν} needed to bring about superposition at fairly large values of $(Nb^2/6a^2)^{1/2}$ indicates that a family of curves for K_f *vs.* $(Nb^2/6a^2)^{1/2}$ in a given pore model are of quite similar shape; that is, the ratio of abscissas for branched and linear chains at the same value of K remains fairly constant over a wide range of K for a given f.

It is also of some interest to inquire if the curves in Figure 1 (or Figure 2) for a given polymer model in the three kinds of cavities can be brought into reasonable coincidence by multiplying the abscissa by a scale factor. The most obvious procedure to try is to adjust

TABLE I
BEHAVIOR OF THE PARTITION COEFFICIENT FOR BRANCHED CHAINS AT THE LIMIT OF SMALL PERMEATION
(PARAMETERS FROM EQ 14 AND 15)

f	ψ	g	ν
1, 2	0.5642	1.0000	
3	0.5415	0.7778	0.1634
4	0.5178	0.6250	0.1824
5	0.4964	0.5200	0.1957
6	0.4775	0.4444	0.2058
7	0.4551	0.3878	0.2139
8	0.4458	0.3438	0.2205
9	0.4325	0.3086	0.2260
10	0.4205	0.2800	0.2308
11	0.4097	0.2562	0.2350
12	0.3997	0.2361	0.2387

the curves to a common initial slope. In Figure 3 we do this for linear chains by plotting K_l against $\lambda R/a$, with λ as in eq 13. Although some degree of agreement among the three curves is apparent (with the curves for the sphere and cylinder much more similar than either is to the curve for the slab), they still diverge markedly when K is less than 0.5. Introduction of arbitrary scale factors to bring about coincidence at some other point, say for $K = 0.5$, would bring the curves fairly close together over a wide range of K. But the basic discrepancy remains; the curves are rather dissimilar in shape.

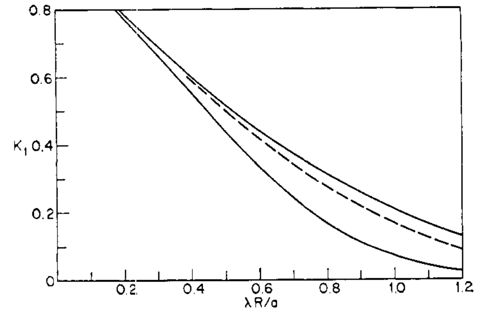

Figure 3. Plots of K for linear polymer chain *vs.* $\lambda R/a$ with λ equal to 1, 2, 3, respectively for slab (lower solid curve), cylinder (dashes), and sphere (upper solid curve).

This not very successful attempt to superimpose the curves by forcing the initial slopes to coincide has a rational motivation indicated in the preceding section. Achievement of superposition in this way would point to the quotient volume/surface as a fundamental dimension that determines the permeability of any cavity. The representation of equilibrium partitioning as largely determined by an internal surface is considered in detail by Giddings, *et al.*[26], for rigid solute molecules of various shapes.[27]

Equilibrium Permeation and Elution Chromatography. In the previous communication,[2] we compared theoretical values of K for linear chains with values deduced from column elution experiments. In making this connection, we assumed that the elution volume—the volume of liquid passed through the column at the appearance of the elution peak due to a homogeneous solute—is given by eq 16, where V_0 is the volume of the

$$V_e = V_0 + KV_i \tag{16}$$

exterior solution phase and V_i is the volume within those pores that can be penetrated by the solvent. This is the basic relation for exclusion chromatography, if K is interpreted as the fraction of the internal volume V_i available to the solute. Obviously, until we specify a molecular model for exclusion, it makes no real difference whether we think of part of the internal volume as completely unavailable to solute and the concentration in the remainder the same as in the mobile phase, or all of the internal volume as equally available with the solute concentration a factor K of that in the mobile phase.

There is an inherent conceptual ambiguity in defining the internal and external volumes; but for practical purposes we can adopt an operational definition. The volume of pure solvent added to a column containing the chromatographic material defines the total volume $V_0 + V_i$ (exclusive of any totally "blind" voids and of the actual volume of the solid material forming, the matrix of pores). To distinguish V_0 from V_i, we can imagine adding to the column, not solvent, but rather a solution of some polymer of such high molecular weight that permeation of voids by solute can be regarded as effectively negligible. Then the solvent, but not the solute, enters the pores in the column packing, and a measurement of the increase of concentration of polymer in the external solution in a static equilibration experiment yields the total volume excluded to polymer. For the determination to be meaningful, it has to be ascertained that there is no specific adsorption of polymer on the porous matrix (in actual experiments this may be a serious complication). This last operation indicates particularly clearly the nature of the ambiguity that is not quite avoided: strictly speaking, the distinctions between V_i and V_0 can never be quite independent of the macromolecular calibrating substance (nor of the size of solvent molecules). Imagine, specifically, that we use very large spherical latex particles. Then, their radius r specifies a distance of closest approach of their centers of mass to all surfaces in the column—for example, the external surface of the beads of the support material—and thus some portion of the *measured* internal volume includes a layer of thickness r in addition to the pores inside the solid matrix. It is conceivable that in some systems this "surface" contribution is not altogether negligible.

Equation 16 follows merely from the assumption of instantaneous equilibration of mobile and stationary phases in the chromatographic column and the requirement that mass be conserved; but it can be simply demonstrated that the equilibrium condition is much more stringent than is necessary. As a thin lamina of solution in the mobile phase moves along the column, exchanging solvent and solute molecules with the sta-

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(26) J. C. Giddings, E. Kucera, C. P. Russell, and M. N. Myers, *J. Phys. Chem.*, 72, 4397 (1968).

(27) In fact, as eq 13 leads us to suspect, the ratios of λ for the various cavity models have nothing to do with the random-flight character of a polymer chain. If the molecule inside the cavity is a rigid sphere of radius r, the distribution coefficient is $(a - r)/a$, $[(a - r)/a]^2$, and $[(a - r)/a]^3$, respectively, for slab, cylinder, and sphere; and the initial slopes of K *vs.* r/a are -1, -2, -3.

tionary phase, the moving zone is in contact momentarily with the stationary phase at each level; and except at the limit of infinitely slow flow, equilibrium cannot be established at all points of the column. But rather than dwell on disequilibrium of the distribution of solute between the two phases, we can consider the experience of a typical solute molecule as it proceeds along the column. If the polymer concentration is very low, entrapment in the stationary phase and release from it are random events governed by independent rate constants. It follows that if the column is sufficiently long (and flow is sufficiently slow) for a molecule to undergo many captures and releases from the stationary phase,[28] the fraction of time it spends trapped depends only on the equilibrium distribution of the species in question between the two phases. Consequently, in what we can term a quasi-equilibrium situation, the average retardation of any species, measured by its elution volume, is amenable to statistical thermodynamic analysis, without regard to kinetics. For flexible-chain polymers this reasoning implies the assumption that hydrodynamic effects in the moving phase do not appreciably distort molecular conformations from their equilibrium distribution.

Though they have not always been appreciated in regard to polymers, there is nothing novel in these ideas; they are basic to chromatography of small molecules that undergo specific interactions with the stationary medium. In particular the correspondence is explicit in the stochastic treatment by Giddings and Eyring[29,30] of adsorption and desorption of solute at reactive sites on the stationary phase. Equivalent results have been obtained by McQuarrie,[31] and the formalism has recently been applied to gel permeation chromatography by Carmichael.[14] In these theories, the trapping and release of a solute species from a given kind of site are determined by two rate constants: the probabilities per unit time k_1, that a molecule in the mobile phase will be trapped, and k_2, that a trapped molecule will be released. Since these events are independent, it is implied that the solution is extremely dilute. For simplicity it is also assumed that the solute is homogeneous and pores are all alike, that the sample is injected at time $t = 0$ as an infinitely thin layer of solution above a volume $V_0 + V_i$ of solvent, that the flow rate through the column is held constant during the experiment, that there is no diffusion along the column, and that all other hydrodynamic and instrumental effects that in practice affect the dispersion of an effluent peak[32] are absent. Thus all solute molecules spend the same time t_0 in the mobile phase and the dispersion of the peak arises from the distribution of total time t_s that a molecule is immobilized in the stationary phase.

For the process defined in this way, the number of entrapments per molecule is governed by a Poisson distribution.[29] This leads to the probability density (eq 17) for finding any given molecule emerging from the column at time $t = t_0 + t_s$, where $I_1(x)$ denotes the modified Bessel function of the first kind.[33] Expanding the Bessel function in an asymptotic series and differentiating, Giddings and Eyring obtained for the maximum in $P(t)$

$$P(t) = \left(\frac{k_1 k_2 t_0}{t_s}\right)^{1/2} [\exp(-k_1 t_0 - k_2 t_s)] I_1[(4k_1 k_2 t_0 t_s)^{1/2}] \quad (17)$$

$$(t)_{\max} - t_0 \approx \frac{k_1 t_0}{k_2}\left(1 - \frac{3}{2k_1 t_0}\right) \quad (18)$$

The stipulated proportionality between time and the volume output of the column gives

$$[(t)_{\max} - t_0]/t_0 = (V_e - V_0)/V_0$$

in terms of the volumes appearing in eq 16; and further, the probabilities k_1 and k_2 are related to the distribution coefficient K by

$$K = k_1 V_0 / k_2 V_i$$

Putting these relations into eq 18 and denoting $k_1 t_0$, the mean number of entrapments per molecule, by $\bar{N}$, we obtain

$$V_e = V_0 \approx K V_i\left(1 - \frac{3}{2\bar{N}}\right) \quad (19)$$

Equation 19 provides a quantitative verification of the intuition that suggests applicability of eq 16; it shows that the assumption of complete equilibrium leads to a fractional error in $V_e - V_0$ of the order of $1/\bar{N}$.

The variance of the distribution in eq 17 is

$$\mathrm{Var}\,(t) = \mathrm{Var}\,(t_s) \approx 2k_1 t_0/k_2^2 = 2\bar{N}/k_2^2$$

Equivalently, in terms of a reduced random variable this is

$$\mathrm{Var}\,(t/t_0) = \mathrm{Var}\,(V/V_0) = t_0^{-2}\,\mathrm{Var}\,(t) \approx 2K^2 V_i^2/\bar{N} V_0^2 \quad (20)$$

where V is the volume removed from the column at time t. Though we use the term "stationary phase" in keeping with the usual language of chromatographic theory, we do not mean to deny the possibility of some mass flow in pores. Such flow may be important in promoting mixing and, thereby, the attainment of equilibrium.

Correlation with Experiment. 1. Determination of the Distribution Coefficient. Granting the correctness of eq 16, we have three routes to determining the distribution coefficient K: (a) from column elution experiments; (b) from static experiments in which the polymer solution and the porous medium are simply mixed and allowed to reach equilibrium; (c) from theoretical calculations, such as we have carried through here, together with independent information on molecular size (which

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(28) In this connection, we remark that the mean residence time of polymer in a conventional gel permeation column experiment is of the order of at least 0.5–1 hr.

(29) J. C. Giddings and H. Eyring, *J. Phys. Chem.*, **59**, 416 (1955).

(30) J. C. Giddings, *J. Chem. Phys.*, **26**, 169 (1957).

(31) D. A. McQuarrie, *ibid.*, **38**, 437 (1963).

(32) J. C. Giddings and K. L. Mallik, *Anal. Chem.*, **38**, 997 (1966).

(33) Equation 17 is correctly normalized but the integral of $P(t)$ over all t is not quite unity since no account is taken of molecules that are not trapped at least once.[29,31]

can be obtained in principle by diffraction experiments). Aside from various practical difficulties and the possibility that the requirements of the idealized elution process we have assumed are not met, failure of a and b to agree means that departures from equilibrium in chromatography are too great for eq 16 to be applicable; and disagreement between a and c of course indicates inadequacy in the equilibrium assumption, in the theoretical pore model, or in both. Two important general comments can be made concerning a and b. First, internal checks on a are possible: V_e is sensibly unaffected by changing the flow rate (which changes t_0 and thus $\bar{N}$) if eq 16 is adequate; and according to eq 20, large departures from equilibrium, making $K^2/\bar{N}$ not completely negligible compared to unity, are bound to affect markedly peak dispersion.[14,26,34] Second, the correspondence between a and b owes nothing to assumptions about pore geometry, statistics of polymer chain conformations, or thermodynamic properties of the polymer–solvent system.

The "absolute" comparison of experimental distribution coefficients with theory will usually prove difficult, and perhaps ultimately unsatisfactory, because the cavities in any actual chromatographic support material are certain to be somewhat nonuniform and more complicated in shape than the rather simple models that are amenable to theoretical analysis. A case in point is provided by the data of Moore and Arrington[11] on elution of linear polystyrene (a series of anionic polymers of narrow distribution with molecular weights ranging from 1×10^4 to 3.5×10^6) in a binary Θ solvent (six parts butanone, one part isopropyl alcohol by volume at 25°) from columns of porous glass. The glass (prepared by Haller[35,36] by dissolving out one phase of a pulverized two-phase glass) has been shown by mercury intrusion measurements and electron microscopy to have remarkably uniform pores. Assuming the pore radii (120 and 900 Å) determined by mercury penetration for the two samples of glass that Moore and Arrington used, we plotted[2] the experimental values of K_1 (determined *via* eq 16 from elution volumes) against R/a and made a comparison with theoretical curves shown in Figure 1. Although agreement with the curve for the slab was excellent, we have to regard the result as coincidental since by no reasonable indulgence of imagination can the pores in the glass be thought of as lamellar. We expect rather than the cylindrical pore model should be more appropriate. The most likely explanation of the data is that the mercury penetration method underestimates the dimensions of nonuniform pores (a wide pore with a smaller entrance is recorded as having the radius of the opening) with the result that the calculated R/a is too large. A degree of nonuniformity that can give such a result is not incompatible with the earlier statement that the pores are unusually uniform. Although the polar solvent was designed to discourage adsorption of polymer on the glass,[11] this effect too would operate in the observed direction—making the apparent K too large.

Even if the insufficiently well-defined structure of real pores makes calculation of K_f unrewarding, the difficulty largely disappears if we require only the comparative behavior of different molecules in the same column. As Figure 2 makes evident, we can ask how the elution volumes of two straight chains of different masses, or of a cruciform molecule and a linear one, differ without having to be greatly concerned as to whether our model for the pores is realistic. Questions of this sort are the matters of greatest importance in assessing chromatographic separations.

2. Does Quasi-Equilibrium Prevail in Ordinary Chromatographic Experiments? We have stated the criteria that must be met if eq 16 is to be valid in terms of the equilibrium distribution coefficient; whether the requisite conditions are actually met has to be decided empirically. At present, there is no consensus as to the predominating mechanism of the separation in gel permeation chromatography. Some authors[3,37–39] have stressed the role of preferential exclusion of large molecules from small pores but others[40–43] have been more concerned with relative diffusion rates for molecules of different size in the stationary "gel" phase. It has been recognized, however, that a single mechanism is unlikely to prevail universally irrespective of operating conditions and of the nature of solute, solvent, and the column material.[39,43,44] By the experimental criteria, considerable data exist to support the contention that a chromatographic regime in which elution maxima are governed solely by equilibrium considerations easy to establish. In the first place, there is the common observation that elution volumes from permeation columns usually show little dependence on flow rate. Further, as Giddings, *et al.*,[26] remark, the width of peaks from efficient columns is too small, despite the various factors that contribute to peak spreading, to be compatible with large deviations from equilibrium.

From the evidence now available, we conclude that exclusion chromatography of chain polymers, as ordinarily carried out, on columns of Haller's porous glass and on porous silica beads[45] conforms to the quasi-equilibrium limit. In their study, Moore and Arrington[11] found that elution volumes for polystyrene in benzene were scarcely affected when the flow rate was varied from 0.1 to 1.0 ml/min in columns 0.5 cm² in cross section. Similarly, Beau, LePage, and de Vries[46] eluted the same polymer-solvent system from silica and found practically no dependence of V_e on linear flow rates (based presumably on the cross section of the

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(34) J. C. Giddings, "Dynamics of Chromatography, Part 1. Principles and Theory," Marcel Dekker, New York, N. Y., 1965.

(35) W. Haller, *J. Chem. Phys.*, **42**, 686 (1965).

(36) W. Haller, *Nature*, **206**, 693 (1965).

(37) J. Porath, *Pure Appl. Chem.*, **6**, 233 (1963).

(38) G. H. Lathe and C. R. J. Ruthven, *Biochem. J.*, **62**, 665 (1956).

(39) T. C. Laurent and J. Killander, *J. Chromatogr.*, **14**, 317 (1964).

(40) A. Polson, *Biochem. Biophys. Acta*, **50**, 565 (1961).

(41) R. L. Steere and G. K. Ackers, *Nature*, **196**, 475 (1962).

(42) W. B. Smith and A. Kollmansberger, *J. Phys. Chem.*, **69**, 4157 (1965).

(43) W. W. Yau and C. P. Malone, *J. Polym. Sci., Part B*, **5**, 663 (1967).

(44) G. K. Ackers, *Biochemistry*, **3**, 723 (1964).

(45) A. J. de Vries, M. LePage, R. Beau, and C. L. Guillemin, *Anal. Chem.*, **39**, 935 (1967).

(46) R. Beau, M. LePage, and A. J. de Vries, *Appl. Polym. Symp.*, in press.

mobile phase) up to about 0.3 cm/sec. At higher flow rates, a gradual decrease in V_e became apparent, but the effect exhibited no definite dependence on the molecular weight of the polymer. It is likely that cellulosic molecules and compact particles such as proteins and viruses can also equilibrate with these inorganic packing materials in times comparable with residence times in chromatographic experiments. In a recent paper, Haller[47] reported that permeation attained 60% of the equilibrium value within 1 min after a dilute solution of a spherical virus of 130 Å radius was mixed with an aqueous suspension of porous glass containing pores of 310 Å radius. After 1 hr, no further significant change in permeation could be detected.[48]

Since the geometrically regular pores dealt with in our theoretical models resemble, more or less, the actual structure of the inorganic glasses, these materials commend themselves for empirical comparison with theory. But even if, as we suggest, elution from such columns is governed by equilibrium partition, it does not follow that the same has to be true of all the heretofore more familiar column packings composed of beads of swollen cross-linked polymer. It is for columns of this latter kind that diffusion has sometimes been assigned the dominant role. The extreme of dependence on diffusion alone is conceivable physically. We can imagine, for example, conditions such that there are no net thermodynamic interactions between free polymer chains and polymer chains that form a very dilute gel network. Then, the equilibrium distribution coefficient is unity; and even though trapped polymer chains might be momentarily restricted by intertwining with the gel network, they still possess, in terms of equilibrium, the conformational freedom allowed outside the gel. In this special circumstance, we would expect all components of a homologous mixture of high polymer species to approach the same average residence time in a sufficiently long column and, therefore, to emerge in a single undifferentiated peak. The only mechanism for separation would, therefore, have to arise from differences in diffusion rates in the stationary phase, which would result in differing apparent values of K for the various solute species for columns of finite length. It follows that there would be an optimum column length (at a given flow rate) for achieving some fractionation of polymer species.

Theoretical conclusions on the importance of nonequilibrium effects in permeation of swollen gels by coiling polymers differ.[43,49] Unfortunately, no realistic model has proved amenable to analysis, and the existing treatments are too abstract to inspire much confidence that they correspond to real systems. Similarly, experimental evidence is inconclusive. Though it does seem that elution volumes are usually insensitive to flow rate in normal column operation, evidence has also been adduced for appreciable nonequilibrium effects.[50]

For compact rigid particles permeating a gel network there is no difficulty in envisioning volume exclusion in the straightforward geometrical sense, even if the gel structure exhibits little or no impenetrable surface—a sphere impinging on a line "sees" a cylindrical forbidden volume of the same radius as itself—and a random-fiber model of the gel, due to Ogston,[51] has been used[26,52] in discussing chromatography of biological macromolecules. In an important experimental study of proteins and viruses on dextran gels, Ackers[44] determined both elution behavior and equilibrium partitioning. He found excellent agreement for tightly cross-linked gels; but for a highly swollen gel with few cross-links, complex effects inconsistent with the idealized description of deviations from equilibrium outlined in the preceding section.

The diversity of experimental results with gels may well reflect differences in structure depending on variations in degree of cross-linking and details of the preparation of these materials. A heavily cross-linked gel[3] that swells by a comparatively small amount in the solvent may have a pore structure resembling the geometrically well-defined, rigid pores that are characteristic of the glasses, but a lightly cross-linked material may be better represented by the conventional picture of an elastic polymer network.

3. Universal Calibration of Chromatographic Columns. Inasmuch as the practical objective of permeation chromatography is the separation of solute species according to molecular size (or mass), a recurrent theme of study is the search for some general molecular-size parameter that determines the position of any elution peak in a chromatogram. In other words, one seeks a means of calibrating a column by passing through it a standard substance for which the value of the relevant parameter is known. This quest is perhaps particularly attractive for work on typical chain polymers since they share the basic structural attribute of a flexible skeleton of covalent bonds. The most elementary assumption to make is that elution is governed simply by the extended length of the chain[7] (or, if one considers only a single structural class such as vinyl polymers, by the number of atoms in the chain backbone). This scheme is perhaps inspired by the idea that passage into a cavity should in some way depend on the relative sizes of the solute molecule and the cavity. Although the elution volume from a given column ought to be a single-valued function of any molecular size parameter of a homologous series of polymer species (at least for the same solvent), several objections can be raised to the molecular contour length: it represents merely the least probable end-to-end distance of a linear chain; it allows in no way for the conformational changes related to thermodynamic interactions between polymer and solvent; it is inapplicable to chains with branches.

Seeking a physically more realistic correlation between elution volume and molecular size, Moore and Arrington[11] assumed that the pore diameter in a column represents a critical size that some molecular dimension must not exceed at any instant when the molecule

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(47) W. Haller, *J. Chromatogr.*, **32**, 676 (1968).

(48) Lest this remark convey an oversanguine implication, we note that Haller found the apparent partition coefficient deduced from elution of the virus to be in disagreement with the value from the static experiment.

(49) J. J. Hermans, *J. Polym. Sci., Part A-2*, **6**, 1217 (1968).

(50) W. W. Yau, H. L. Suchan, and C. P. Malone, *ibid., Part A-2*, **6**, 1349 (1968).

(51) A. G. Ogston, *Trans. Faraday Soc.*, **54**, 1754 (1958).

(52) L. M. Siegel and K. J. Monty, *Biochim. Biophys. Acta*, **112**, 346 (1966).

is capable of entering the stationary phase. From their data on polystyrene on porous glass they concluded that molecules with a mean maximum projection on a line[21,53] equal to the pore diameter give $K = 0.5$.

Benoit and coworkers[10,12] have recently proposed that elution is governed by the product of intrinsic viscosity and molecular weight for any polymer, linear or branched. By making two assumptions, we can show that this empirical suggestion is in accord with our theoretical calculations of equilibrium permeation by branched flexible chains. We assume that the intrinsic viscosity for a linear coiling polymer is given by the familiar relation[54]

$$[\eta]_{\text{lin}} = \Phi R^3/M \tag{21}$$

where Φ is a constant, independent of the specific nature of the polymer for reasonably high molecular weights. For star branched chains we also accept an approximate expression given by Zimm and Kilb,[55] relating the intrinsic viscosities of linear and branched chains of the same mass in a Θ solvent

$$[\eta]_{\text{br}} = [\eta]_{\text{lin}} g^{1/2} \tag{22}$$

Combining eq 21 and 22, we have

$$\begin{aligned}[\eta]_{\text{br}} M &= [\eta]_{\text{lin}} g^{1/2} M = \Phi(R^3)_{\text{lin}} g^{1/2} \\ &= \Phi[(Nb^2/6)^{1/2} g^{1/6}]^3 \qquad (22)\end{aligned}$$

Hence, species with the same value of $(Nb^2/6)^{1/2} g^{1/6}$ have the same value of $[\eta]_{\text{br}}M$ and (to the approximation indicated by Figure 2) the same value of the distribution coefficient K on a given chromatographic column. Consequently, if eq 16 holds, they will elute at the same point.

The Einstein viscosity relation for a dilute suspension of spheres can be written as

$$[\eta]M = (\pi N_{\text{A}}/30)R_\eta^3$$

where N_{A} is Avogadro's number, R_η is the radius of the sphere, and $[\eta]$ is expressed in conventional units of grams per 100 ml. Applied to polymer chains, this expression defines an effective hydrodynamic molecular radius proportional to $([\eta]M)^{1/3}$. The finding that this product determines the elution volume for a variety of linear and branched polymers has led to speculation[12] that the hydrodynamic size governs the entrapment process in gel chromatography. However, our calculations for random flight chains show that this conclusion is not necessary. Though our results do indeed correlate quantitatively with the hydrodynamic radius, they are obtained entirely from equilibrium thermodynamics.

It seems worth emphasizing here that establishing the validity of any general calibration parameter for gel permeation depends crucially on tests involving the behavior of more than a series of typical linear polymers. In a Θ solvent, the various statistical averages of chain dimensions—root-mean-square end-to-end distance, root-mean-square radius of gyration and others[53]—are all proportional to $N^{1/2}b$ for a linear chain; and so is R_η if eq 21 is correct. Hence, if there is found to be a good correlation of one of these quantities with elution volume, any of the others has to work equally well. The conformation of polymers in good solvents cannot correspond exactly to random flight statistics; but the simplest, and most familiar, approximation of an expansion of all unperturbed dimensions by a common factor preserves the proportionality among the averaged dimensional quantities. On the other hand, if the approximation of uniform chain expansion is inadequate, or if Φ is not independent of the polymer and solvent, there can be reason in principle for suggesting that one measure of molecular size is better than others in correlating elution data on linear chains. For example, Ptitsyn and Eizner[56] have formulated a theory in which Φ in eq 21 depends on chain expansion. But such deviations from the simplest view of hydrodynamic behavior are still a matter of dispute, both theoretically and experimentally, and for many systems cannot be expected to be very large. It seems fair to conclude that elution data available so far on linear polymers provide no basis for choosing between R and R_η as a possibly appropriate parameter for calibrating a gel permeation column; it is only the results on branched chains that can be decisive in this respect.

Conclusion

To summarize, we have in this paper (a) calculated the equilibrium distribution of a star-branched flexible polymer species between a solution of indefinitely large volume and voids of the same order in size as the domain of the dissolved solute molecule; (b) applied simple chromatographic theory to show that the elution volume from a permeation column should be determined under quite broad conditions by the equilibrium distribution of solute between mobile and stationary phases; (c) shown that the equilibrium results in fact correlate very well with the suggestion that the hydrodynamic radius of the polymer molecule is the determining parameter in the elution volume.

It must be remembered that our equilibrium calculations are limited, for simplicity, to the ideal case where the effect of restricting chain conformations to those that fit inside a void is purely a decrease in entropy by $k \ln K$ per mole, k denoting Boltzmann's constant. In a good solvent, the permeation of any given polymer species is further diminished by two effects: the entropy change attending transfer of a chain to a void is increased because the molecule, when unhindered by the cavity, is expanded (on the average) beyond random flight dimensions; and there is an enthalpy change arising from the greater density of intramolecular contacts in a chain confined to a cavity than in a chain outside. The first effect is accommodated easily in the framework of the present derivation if we accept the

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(53) M. V. Volkenstein, "Conformational Statistics of Polymeric Chains," Interscience Publishers, New York, N. Y., 1963, p 181.

(54) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, Chapter 14.

(55) B. H. Zimm and R. W. Kilb, *J. Polym. Sci.*, **37**, 19 (1959).

(56) O. B. Ptitsyn and Yu. E. Eizner, *Zh. Tekh. Fiz.*, **29**, 1117 (1959).

uniform-expansion approximation for the polymer chain[45]. One way of treating the second is indicated in a derivation by Meier, which—although it deals with a different problem—resembles our work in both spirit and form.[57]

The relation between equilibrium permeation and column chromatography points to the importance of equilibrium measurements as an aid to understanding the chromatographic process.[58] Though equilibrium measurements reveal nothing about peak spreading, nor do they give the values of the kinetic constants k_1 and k_2 for trapping and release of polymer chains in the column, they do obviously fix the ratio of the constants. In view of the facility of equilibrium measurements—and the fact that Ackers[44] made such a study on proteins a few years ago—it is surprising that no work on equilibrium permeation of chain polymers seems to have been published.

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Appendix

Systematic Calculations for Branched Chains in Spherical and Slab-Shaped Cavities. The product of sines in the integrand of eq 4 can be transformed to sums and differences of sines or cosines by repeated application of trigonometric identities: *e.g.*, with three sines

$$\sin A \sin B \sin C = \tfrac{1}{2} \sin A [\cos (B - C) - \cos (B + C)] = \tfrac{1}{4}[\sin (A + B - C) + \sin (A - B + C) + \sin (-A + B + C) - \sin (A + B + C)] \quad \text{(A1)}$$

Integration of the individual terms is obvious, but for computations for any number of branches f, we have to systematize the results. A little experimentation with the general integral

$$I = \int_0^1 \sin (p_1 \pi x) \sin (p_2 \pi x) \ldots \sin (p_j \pi x)\, \mathrm{d}x$$

where p_1, p_2, etc., are *odd integers*, serves to establish the following presentation. When j is *even*, we can write

$$I = (-1)^{j/2} \left(\frac{1}{2}\right)^{j-1} [Z_j - Z_{j-1} + Z_{j-2} - \ldots + \tfrac{1}{2}(-1)^{j/2} Z_{j/2}] \quad \text{(A2)}$$

where each term Z_{j-i} denotes the sum of $\binom{j}{j-i}$ functions $\zeta(w)$ obtained by permuting $j - i$ plus signs and i minus signs to form all different linear combinations of the indices p in

$$w = \pm p_1 \pm p_2 \pm \ldots . \pm p_j \quad \text{(A3)}$$

and then defining

$$\zeta(w) = \begin{cases} 0 & (w \neq 0) \\ 1 & (w = 0) \end{cases} \quad \text{(A4)}$$

Only the signs of the p's, not the subscripts, are to be permuted. When j is *odd*, the result is

$$I = (-1)^{(j-1)/2} \left(\frac{1}{2}\right)^{j-1} [H_j - H_{j-1} + H_{j-2} - \ldots + (-1)^{(j-1)/2} H_{(j+1)/2}] \quad \text{(A5)}$$

with H_{j-i} representing sums of terms $\eta(w)$ obtained as before by permuting plus and minus signs to form the possible w's, but with $\eta(w)$ now defined by

$$\eta(w) = 2/\pi w \quad \text{(A6)}$$

The evaluation of $(K_f)_{\text{sphere}}$ falls into a similar pattern. Obviously, each f-fold product of sines in eq 7 can be transformed into a sum of sines or cosines as in eq A1 and the integral of each term evaluated. That is, we convert the integral

$$I' = \int_0^1 \sin (p_1 \pi x) \sin (p_2 \pi x) \ldots \sin (p_j \pi x)\, \mathrm{d}x / x^{j-2}$$

to a sum of integrals of the form

$$\int_0^1 \frac{\sin (\gamma \pi x)}{x^{j-2}}\, \mathrm{d}x$$

for j odd, and

$$\int_0^1 \frac{\cos (\gamma \pi x)}{x^{j-2}}\, \mathrm{d}x$$

for j even. The constants γ are integers. Then integration by parts, repeated $j - 3$ times, reduces these integrals to expressions including trigonometric terms and a term containing Si $(\gamma\pi)$. Although Si $(\gamma\pi)$ is infinite, each divergent term in the final result cancels exactly with another of opposite sign. This complication can be avoided by combining terms at an intermediate stage.

The computation of the integrals I' can be organized in much the same fashion as for I in eq A2–A6. Each integral can be put in the form

$$I' = (-1)^{j/2} \left(\frac{1}{2}\right)^{j-1} [M_j - M_{j-1} + \ldots + \tfrac{1}{2}(-1)^{j/2} M_{j/2}]$$

for j even, or

$$I' = (-1)^{(j-1)/2} \left(\frac{1}{2}\right)^{j-1} [N_j - N_{j-1} + \ldots + (-1)^{(j-1)/2} N_{(j+1)/2}]$$

for j odd, where M_{j-i} and N_{j-i} are respectively sums of $\binom{j}{j-i}$ quantities $\mu(w)$ and $\nu(w)$ obtained by permuting signs in eq A3 and then using

$$\mu(w) = \frac{(-1)^{(j+2)/2}}{(j-3)!} (w\pi)_j^{-3} \operatorname{Si} (w\pi)$$

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(57) D. J. Meier, *J. Phys. Chem.*, **71**, 1861 (1967). From discussions occasioned by the initial presentation of our results, we have learned that in unpublished work both Dr. Meier and Dr. T. Alfrey have independently treated the equilibrium of coiling polymers in simple cavities as a mathematical problem in restricted diffusion.

(58) J. B. Carmichael, *Polym. Preprints*, **9**, 572 (1968).

for $j = 4, 6, \ldots,$ and

$$\nu(w) = \frac{(-1)^{(j+1)/2}}{(j-3)!}(w\pi)^{j-3}\,\mathrm{Si}\,(w\pi)$$

for $j = 3, 5, \ldots.$ Obviously, for $j = 1$, we have

$$I' = (-1)^{p_1+1}/p_1\pi$$

and for $j = 2$

$$I' = I = {}^1/_2$$

In the calculation for the sphere, the indices in eq A3 can assume all integer values.

To compute K_f we use eq 4 for the slab or the equivalent expression for the sphere. The integral for each allowed combination of f indices p, q, etc., is obtained with the aid of the above development, and then multiplied by the exponential factor and by the number of distinguishable orderings of the given indices—*e.g.*, $f!$ when p, q, etc., are all different. It will be noted that the rule given in eq A4 causes the integral over the product of f sines, and thus the entire term in eq 4, to vanish for certain combinations of indices. Thus, with $f = 4$ and p, q, r, s equal to 1, 1, 3, 7, there are no linear combinations of the four numbers that equal zero; and so all the $\zeta(w)$, and the integral I, vanish. Equation A6 ensures that none of the integrals in eq 4 vanishes when f is odd. The corresponding integrals for the spherical cavity are all nonvanishing for both even and odd f.

Generally, calculations of K_f for branched chains require retention of rather large numbers of the final series expansion in powers of exponentials. For instance, to calculate K_4 in a slab cavity to three significant figures requires at least the contributions from all permitted combinations of indices for $p^2 + q^2 + r^2 + s^2 \leq 140$ when $(Nb^2/6a^2)^{1/2} = 0.2$. As K_4 decreases, the number of terms needed falls rapidly; the same precision requires terms with $p^2 + q^2 + r^2 + s^2$ up to 76 and up to 36, respectively, for $(Nb^2/6a^2)^{1/2}$ equal to 0.4 and 0.5. Plainly, extensive calculations call for a computer.

As we remarked earlier, the integral in eq 10 for the cylindrical cavity apparently cannot be resolved into a sum of integrals that are expressible analytically, except in the trivial case $f = 2$. Consequently, for numerical calculations we resorted to Gauss quadratures, expressing the integrand of eq 10 as an f-fold sum and integrating successive terms with respect to r'.

Calculations for K near Unity. The slab is the simplest case. We can rewrite eq 11 in the form

$$(K_f)_{\mathrm{slab}} = \int_0^1 \left\{1 - \sum_{p=0}^{\infty}(-1)^p\left[\mathrm{erfc}\left(\frac{2p+1-x}{2u}\right) + \mathrm{erfc}\left(\frac{2p+1+x}{2u}\right)\right]\right\}^f \mathrm{d}x \quad \text{(A7)}$$

where

$$u = (Nb^2/6a^2f)^{1/2} = (nb^2/6a^2)^{1/2}$$

$$x = z'/a$$

Expansion of the series integrand by the multinomial theorem leads to integrals of various combinations of products of powers of erfc functions. Most of these contribute nothing of order u to K. For instance, one of the integrals appearing in the expansion of eq A7 is

$$W = \int_0^1 \mathrm{erfc}\left(\frac{1+x}{2u}\right)\mathrm{d}x = 2u\int_{1/2u}^{1/u}\mathrm{erfc}(\xi)\,\mathrm{d}\xi$$

Integration by parts gives

$$\mathrm{ierfc}\; y \equiv \int_y^{\infty}\mathrm{erfc}(\xi)\,\mathrm{d}\xi = \frac{1}{\sqrt{\pi}}e^{-y^2} - y\,\mathrm{erfc}(y) \quad \text{(A8)}$$

By the definition of ierfc, we have

$$W/2u = \mathrm{ierfc}(1/2u) - \mathrm{ierfc}(1/u) \quad \text{(A9)}$$

Introducing the last equality of eq A8, we find that the right-hand side of eq A9 approaches zero as u becomes small. Like reasoning establishes that integrals with integrands containing any power of $\mathrm{erfc}[(2p + 1 + x)/2u]$ all vanish as u vanishes.

In fact, the only integrals that contribute anything to the linear dependence of K on u are those with nothing but a power of $\mathrm{erfc}[(1 - x)/2u]$ in the integrand. It follows that

$$\lim_{u\to 0}(K_f)_{\mathrm{slab}} = \lim_{u\to 0}\int_0^1\left[1 - \mathrm{erfc}\left(\frac{1-x}{2u}\right)\right]^f \mathrm{d}x = \lim_{u\to 0}\left\{2u\int_0^{1/2u}[\mathrm{erf}(\xi)]^f\,\mathrm{d}\xi\right\} \quad \text{(A10)}$$

For $f = 1$, eq A10 corresponds to the result[25] derived by considering a linear chain in a semiinfinite region bounded by a plane.

An integration by parts gives

$$\int_0^{1/2u}[\mathrm{erf}(\xi)]^f\,\mathrm{d}\xi = \frac{1}{2u}\left[\mathrm{erf}\left(\frac{1}{2u}\right)\right]^f - \frac{2f}{\sqrt{\pi}}\int_0^{1/2u}\xi[\mathrm{erf}(\xi)]^{f-1}e^{-\xi^2}\,\mathrm{d}\xi \quad \text{(A11)}$$

and therefore as u^{-1} approaches infinity, we have

$$\lim_{u\to 0}(K_f)_{\mathrm{slab}} = (1 - 2u)\frac{2f}{\sqrt{\pi}}\int_0^{\infty}\xi[\mathrm{erf}(\xi)]^{f-1}e^{-\xi^2}\,\mathrm{d}\xi \quad \text{(A12)}$$

The substitution $\xi^2 = t$ then leads to ψ in eq 14. Another integration by parts (of eq A12) gives an alternative form

$$\psi = \frac{2f^{1/2}(f-1)}{\pi}\int_0^{\infty}[\mathrm{erf}(\xi)]^{f-2}e^{-2\xi^2}\,\mathrm{d}\xi$$

The error function representation of K_f for the cylindrical cavity is

$$(K_f)_{\mathrm{cyl}} = 2\int_0^1 x(1 - v)^f\,\mathrm{d}x \quad \text{(A13)}$$

where

$$v = \frac{1}{x^{1/2}}\left\{\mathrm{erfc}\left(\frac{1-x}{2u}\right) + \frac{(1-x)u}{4x}\,\mathrm{ierfc}\left(\frac{1-x}{2u}\right) + \ldots\right\} \quad \text{(A14)}$$

and u is defined as above in eq A7. In this case v, unlike the corresponding expression in eq A7, cannot be written as a simple series;[18g] but for our purpose we need only the first term. Introducing the variable ξ as in eq A10, we have from A13 and A14

$$(K_f)_{\text{cyl}} = 4u\int_0^{1/2u}(1 - 2u\xi)[1 - (1 - 2u\xi)^{-1/2}\operatorname{erfc}(\xi) + \ldots]^f\,\mathrm{d}\xi$$

and, by using the binomial theorem twice and rearranging terms

$$(K_f)_{\text{cyl}} = 4u\int_0^{1/2u}(1 - 2u\xi)\{\operatorname{erf}(\xi) - u\xi[1 - \operatorname{erf}(\xi)] + \ldots\}^f\,\mathrm{d}\xi$$

$$= 4u\int_0^{1/2u}\left\{[\operatorname{erf}(\xi)]^f - 2u\xi[\operatorname{erf}(\xi)]^f + fu\xi\{[\operatorname{erf}(\xi)]^f - [\operatorname{erf}(\xi)]^{f-1}\} + \ldots\right\}\mathrm{d}\xi$$

Integrating each term in the expanded form by parts, we note that the second term of the integrand gives

$$8u^2\int_0^{1/2u}\xi[\operatorname{erf}(\xi)]^f\,\mathrm{d}\xi = 1 - \mathrm{O}(u^2)$$

and that the later terms together affect K_f only as u^2 Therefore, using eq A11, we recover eq 13 with $\lambda = 2$.

For the spherical cavity, we can write

$$(K_f)_{\text{sphere}} = 3\int_0^1 x^2(1 - v)^f\,\mathrm{d}x$$

where now

$$v = \frac{1}{x}\sum_{p=0}^{\infty}\left\{\operatorname{erfc}\left(\frac{2p + 1 - x}{2u}\right) - \operatorname{erfc}\left(\frac{2p + 1 + x}{2u}\right)\right\}$$

Again only one term from the series for v contributes to the linear approximation to K_f. Substitution of ξ and use of the binomial theorem, as before, leads to

$$(K_f)_{\text{sphere}} = 6u\int_0^{1/2u}\left\{[\operatorname{erf}(\xi)]^f - 4u\xi[\operatorname{erf}(\xi)]^f - 2fu\xi\{[\operatorname{erf}(\xi)]^f - [\operatorname{erf}(\xi)]^{f-1}\} + 4u^2\xi^2[\operatorname{erf}(\xi)]^f + \ldots\right\}\mathrm{d}\xi \quad \text{(A15)}$$

The first, second, and fourth terms of the integrand in eq A15 give the linear dependence of K_f on u. All other parts of the expansion can be grouped, like the third term, to cancel upon integration, except for quantities of order u^2. Finally, eq 13 with $\lambda = 3$ is recovered.

The computation of ψ in Table I was done by Gauss–Laguerre quadratures. The integral is already of the standard form which is approximated by a polynomial

$$\int_0^{\infty} e^{-x}f(x)\,\mathrm{d}x \approx \sum_{i=1}^{N} A_i f(x_i)$$

The x_i and weighting factors A_i were taken from the tables of Stroud and Secrest.[59] The integral for each value of f was calculated by both 24-point and 32-point summations. Results agreed to at least the four significant figures given in the table.[59a]

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(59) A. H. Stroud and D. Secrest, "Gaussian Quadrature Formulas," Prentice-Hall, Englewood Cliffs, N. J., 1966.

(59a) NOTE ADDED IN PROOF. Since this paper was submitted for publication, a relevant experimental study by W. W. Yau, C. P. Malone, and S. W. Fleming [*J. Polym. Sci., Part B*, **6**, 803 (1968)] has appeared. Using linear polystyrene in chloroform and both a polystyrene gel and a porous glass as chromatographic media, these authors have made comparative measurements, such as we have suggested, of column elution and static equilibrium. For the porous glass, their results are in conformity with the view that the equilibrium constant K should govern elution according to eq 16. More complex behavior is evident in the case of the polystyrene gel, with V_e showing marked dependence on flow rate and nonlinear variation with K for molecular weights above 100,000. Unfortunately, the two chromatographic systems are characterized by a very great difference in effective pore size: the glass entirely excludes polystyrene of molecular weight above 50,000, and thus a direct comparison with the elution behavior of the high molecular weight polymer from the gel is frustrated. Despite obvious discrepancies with predictions from simplified models, experiments such as these lend strong support to the notion that equilibrium partitioning of solute between a mobile phase and a phase in small voids or channels is an important and often dominant factor in exclusion chromatography of conventional chain polymers. It will be plain to the reader that our inclination to this view is more decided now than it was at the time of our previous discussion.[2]