Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Gel Permeation Chromatography and Thermodynamic Equilibrium

Edward F. Casassa[a]
[a] MELLON INSTITUTE AND DEPARTMENT OF CHEMISTRY, CARNEGIE-MELLON UNIVERSITY, PITTSBURGH, PENNSYLVANIA

To cite this Article Casassa, Edward F.(1971) 'Gel Permeation Chromatography and Thermodynamic Equilibrium', Separation Science and Technology, 6: 2, 305 — 319
To link to this Article: DOI: 10.1080/00372367108058964
URL: http://dx.doi.org/10.1080/00372367108058964

SEPARATION SCIENCE, 6(2), pp. 305–319, April, 1971

Gel Permeation Chromatography and Thermodynamic Equilibrium*

EDWARD F. CASASSA

MELLON INSTITUTE AND DEPARTMENT OF CHEMISTRY
CARNEGIE-MELLON UNIVERSITY
PITTSBURGH, PENNSYLVANIA 15213

Summary

Although gel permeation chromatography is firmly established as a technique for investigating heterogeneity in synthetic polymers, the character of the chromatographic process—whether it is dominated by diffusion, by flow effects, or by an equilibrium partitioning of polymer between the mobile phase and the micropores in the column packing—is still disputed. General chromatographic theory supports the idea that under ordinary experimental conditions the equilibrium distribution of a solute determines the position of its elution peak in the chromatogram. Statistical mechanical calculations of distribution coefficients for linear and branched polymer chains and idealized pores of simple geometry lead to predictions in good accord with some experimental findings.

GEL PERMEATION AND CHROMATOGRAPHIC THEORY

In the decade or less since gel permeation chromatography (GPC) was first proposed as a general means for separation of synthetic polymers according to molecular weight, suitable instrumentation has become widely available and the method has attained status as the most popular one for analytical polymer fractionation. Although the practical success, first with compact biological macromolecules and more recently with typical flexible chain polymers, that has amply demonstrated that separation is effected according to molecular size has also spurred inquiry into the physical basis of the separation, the

* Presented at the ACS Symposium on Gel Permeation Chromatography sponsored by the Division of Petroleum Chemistry at the 159th National Meeting of the American Chemical Society, Houston, Texas, February, 1970.

mechanism responsible remains a matter of some dispute. Theoretical treatments have been variously concerned with diffusion processes (*1, 2*), hydrodynamic effects (*2–5*), and thermodynamic equilibrium (*6–11*). Perhaps the only point of general agreement is that the mechanisms proposed are not mutually exclusive: the question is not what effect occurs but rather which one is dominant (*1, 2, 4, 12*).

It is attractive to suppose that a process at or (more precisely) near thermodynamic equilibrium is operative simply because a rigorous theoretical treatment will be inherently simpler than for a mechanism limited by a transport process. Furthermore, even if such optimism should be quite unjustified, it can be argued that the equilibrium situation retains significance as the asymptotic limit to which real behavior must tend when a chromatographic column is operated at extremely small flow rate.

To gain some idea of the applicability of an equilibrium model for GPC, we can turn to the general theory of chromatography. Of the several ways of treating chromatographic processes, the stochastic theory proposed by Giddings and Eyring (*13*), elaborated upon by Giddings (*14*) and by McQuarrie (*15*), and applied to GPC by Carmichael (*16*) is particularly appropriate. The original theoretical model is designed to describe adsorption chromatography. Hence, the description proceeds in terms of a column filled with a granular packing material bearing identical surface sites (constituting a stationary phase) capable of adsorbing a solute reversibly from solution. The interstices between granules, initially filled with solvent, constitute the mobile phase. At a certain moment, a narrow band of solution is introduced at the top of the column. This is followed by more solvent as liquid is withdrawn from the bottom of the column at a steady rate and the solution zone passes down through the column, exchanging solute with the stationary phase. Three assumptions are made: (a) that a molecule of a particular solute species in the liquid phase has a certain fixed probability k_1 per unit time of being adsorbed on a surface site, (b) that a molecule on the surface has a fixed probability k_2 per unit time of escaping into the mobile phase, and (c) that there is no net diffusion of solute molecules in the direction of the column axis while they are in the mobile phase. The first assumption implies that the adsorption isotherm is linear—the solution is so dilute that solute molecules do not interact and there is no multiple adsorption on sites. It further implies that the absorption is not diffusion controlled. This requirement is obviously not physically realizable since the prob-

ability for adsorption of a given molecule can hardly be independent of its distance from a reactive site, but a time-averaged k_1 will be the same for each solute molecule if the residence time in the column is long enough to permit the molecule to undergo a large number of adsorptions and desorption steps. Assumptions (a) and (b) require that absorption sites do not interact—that occupation of a site does not affect k_1 and k_2 at any other site. Assumption (c) means that every solute molecule spends the same time t_0 in the mobile phase as it passes through the chromatographic column. The theory thus neglects various flow effects, as well as longitudinal diffusion, that in real columns contribute to the spreading of chromatographic peaks. The peak shape predicted by the theory is accounted for solely by the probability distribution of the total time t_s that a solute molecule spends absorbed as it pursues its course down the column, alternately in the mobile phase and in the stationary (adsorbed) phase. The object of the stochastic theory is to calculate this distribution as a function of parameters k_1, k_2, and t_0. If the rate of withdrawal of fluid from the column is held constant, $t_0 + t_s$ is proportional to the volume eluted when molecules experiencing a given t_s emerge, and the probability of t_s plotted against t_s for a solute species has the shape of the elution curve—i.e., concentration versus elution volume.

It will be recognized that the foregoing description of a model for adsorption chromatography requires only minimal verbal changes to apply equally to GPC. Now we have a column packing that contains, in place of adsorption sites, microscopic voids, for our purposes of the same order of size as macromolecules and (for simplicity) assumed to be of identical size and shape. The stationary phase is the volume V_i of the part of the column inside micropores and accessible to solvent. All we then have to do is replace the word "adsorption" by the phrase "entrapment in micropores" and recognize that in GPC, unlike adsorption chromatography, the solvent—consisting of small molecules and, therefore, most easily trapped in voids—is retarded in the column relative to macromolecular solutes.*

The mathematical problem is a standard one in probability theory. Interestingly, the result of the calculation is an elution curve that departs somewhat from a Gaussian—it has a positive skew. However,

* Since the name "gel permeation" has been canonized by general acceptance, we use it freely here to designate a kind of chromatography, without meaning to imply that the column packing must be a gel in any conventional sense of the word.

our concern here is not with the details of the theoretical curve shape but primarily with the position of the maximum in the peak along the time (or volume) axis. The result we require is (*13*)

$$V_e \approx V_0 + KV_i(1 - 3/2\bar{N}) \tag{1}$$

where V_e is the volume eluted when the peak maximum appears (henceforth called simply the elution volume) and V_0 is the volume of the mobile phase. Then, $V_0 + V_i$ is the total volume of the column except for the volume of the solid matter in the packing and the volume of "blind" pores that cannot be permeated by solvent. Certain constants are gathered in K,

$$K = k_1V_0/k_2V_i \tag{2}$$

and

$$\bar{N} = k_1t_0 \tag{3}$$

is the mean number of entrapments suffered by a solute molecule in its passage through the column. Equation (1) is an asymptotic relation for $\bar{N}$. Recalling that the mean residence time of a molecule in a GPC column is of the order of a half hour or longer in a conventional experiment, we surmise that this condition is adequately fulfilled. Equation (1) also implies that at the start of the experiment—the moment when withdrawal of liquid from the column begins—the solute is all in the mobile phase. However, the only difference for the alternative extreme of all solute adsorbed at the beginning is the unimportant replacement of $\frac{3}{2}$ in the second term by $\frac{1}{2}$ (*13*).

The obvious, but crucial, deduction from Eq. (1) is that if $\bar{N}$ is sufficiently large, we can write

$$V_e = V_0 + KV_i \tag{4}$$

without appreciable error. Consequently, under realistic experimental conditions, the elution volume V_e from a given column is expected to be insensitive to the flow rate, which of course determines the time t_0, hence $\bar{N}$.

As we expect intuitively, the dispersion of an elution peak does not share this insensitivity to flow rate. Another result of the statistical theory is that the standard deviation of t_s is proportional to $\bar{N}^{1/2}$ (i.e., to $t_0^{1/2}$) for large $\bar{N}$. However, the standard deviation of the ratio t_s/t_0 is asymptotically proportional to $1/\bar{N}^{1/2}$. These relations conform to the essential requirement in a chromatographic separation that increasing the column length at constant flow rate improves resolution

of multiple peaks, even though each peak becomes broader with increasing t_0.

Equation (4) can be regarded as the basic relation for GPC. The constant K as defined in the theory is the ratio of the two rate constants for passage of solute to and out of the stationary phase; hence it is the equilibrium constant, the distribution coefficient, for the partitioning of solute between mobile and stationary phases. Just as in the distribution of a solute between dilute macroscopic phases, K is the ratio of solute concentrations in the two phases; and $-RT \ln K$ represents the free energy change for the process of transporting a mole of solute in its standard state in one phase to its standard state in the other. The possibility is thus afforded of applying the conventional methods of statistical mechanics to simple models for entrapment of solute in pores to deduce K and thus to predict elution volumes from Eq. (4) if V_0 and V_i are known. In general V_0 and V_i can be determined experimentally: the former represents the elution volume for solute molecules so large that they do not penetrate the pores appreciably ($K = 0$), and $V_i + V_0$ is the elution volume for small molecules that penetrate the voids as easily as the solvent does (so that $K = 1$—as would be expected for radioactively labeled solvent).

As the preceding discussion indicates, equilibrium theory may yield important information about gel chromatography—information on the relation of molecular conformation and pore geometry to the elution volume. However, it cannot be expected to reveal anything about peak spreading since the dynamics of molecular entrapment that determine the individual rate constants k_1 and k_2 do not enter into the equilibrium calculations.

CALCULATION OF THE SOLUTE DISTRIBUTION

We have already stipulated that the solution passing through the chromatographic column must be so dilute that interactions between solute molecules are negligible. Then, the equilibrium constant K can be written formally as a ratio of configuration integrals for one molecule:

$$K = \frac{\int \cdots \int e^{-U_s\{q\}} d\{q\}}{\int \cdots \int e^{-U_0\{q\}} d\{q\}} \tag{5}$$

where $U_s\{q\}$ represents an energy associated with a set of spatial coordinates $\{q\}$ that defines a solute molecular conformation in the stationary phase and $U_0\{q\}$ represents the energy as a function of the

requisite coordinates in the mobile phase.* For a spherical molecule appropriate coordinates would be the three needed to locate the center of mass; for a rigid asymmetric molecule, the coordinates of the center of mass plus the angles determining orientation; and for a flexible polymer chain, coordinates of the center of mass, or a chain end, plus coordinates of each segment relative to this reference point. The symbol $d\{q\}$ denotes a differential element of the configuration space of as many dimensions as are needed to describe a molecular conformation. The two multiple integrations are carried out over equal macroscopic volumes in physical space, e.g., a unit volume of solution and the same volume of space inside pores constituting the stationary phase (*10*).

Evaluation of the integrals in Eq. (5) is in general impossible, and further progress depends on placing drastic limitations on the energies $U_s\{q\}$. Here we let these energies have but two values, zero (or any fixed finite value) and infinity. When U_s is zero, the conformation is allowed; when U_s is infinite, the conformation is forbidden. Thus we represent the walls of a pore as rigid boundaries which a molecule cannot pass. Any conformation intersecting the boundary has infinite energy and is thereby excluded. Adsorption of solute, which would imply minimum energy for conformations contiguous to the boundary surface, is also excluded. For nonrigid molecules the assumption that all allowed conformations have the same energy means that intramolecular interactions are ignored. For flexible long-chain molecules, the case of particular interest, this means that the polymer–solvent system is at its characteristic "theta temperature," at which the second virial coefficient in the osmotic equation of state is zero (and at which phase separation occurs if the polymer is of infinite molecular weight) (*18*). At the theta point, the molecular conformation of a long-chain polymer can be described by random flight statistics, a fact that enormously facilitates mathematical analysis.

With the above limitations on permissible energies, the distribution coefficient K is expressed as a ratio of volumes in configuration space; and thus $R \ln K$ is a standard entropy change per mole of solute. For rigid spherical molecules, the ratio of configuration integrals reduces to a ratio of volumes in physical space. This straight-

* In more precise language, these energies are potentials of mean force (*17*) between solute molecules. They include implicitly the effects of solute–solvent and solvent–solvent interactions.

forward volume-exclusion picture of GPC has long been used to account for the elution behavior of compact biological macromolecules. For example, a spherical solute molecule of radius r does not interact with a wall when its center of mass is farther than a distance r from the wall, and it can approach no closer than r. Consequently, in a slab-shaped space between parallel (infinite) planes a distance $2a$ apart, the equilibrium constant is $(a - r)/a$, the volume "seen" by the sphere divided by the actual volume of a cavity. The same sphere in a long cylindrical cavity of radius a and inside a hollow sphere of radius a gives K equal, respectively, to the square and cube of $(a - r)/a$. Various geometrical situations for exclusion of rigid molecules have been investigated (*6–8, 10, 19*). The calculations are sometimes intricate but the principle remains the same; the calculated K represents the fraction of the void volume effectively available to solute.

The exclusion of a flexible polymer chain from part of the volume inside micropores is perhaps less obvious. Because such a molecule is allowed to assume any shape, however tortuous, no part of the actual volume of a pore is excluded from occupancy by any part of the polymer chain; but since configurations that intersect the walls of a void are disallowed, the number of permitted configurations per unit volume is still decreased by proximity to a wall. In other words, although the geometry of the situation is more complicated than for the rigid sphere model, it is still true that the region of *configuration* space available to the solute is reduced by the presence of an impenetrable boundary.

These ideas can be illustrated concretely by considering the least specific model for a micropore: whatever the shape of the pore, we let it be characterized simply by the ratio σ of its surface to its volume and assume that solute molecules intersect with the surface as if it were a plane of infinite extent. Therefore, a spherical solute of molecular radius r, rolling over the surface of any cavity, will have its center of mass excluded from a fraction σr of the volume of the cavity, and then according to Eq. (5), the distribution coefficient is given by

$$K = 1 - \sigma r \tag{6}$$

Representing σ by $1/a$, $2/a$, and $3/a$, for slab-shaped, cylindrical, and spherical cavities, respectively, we obtain expressions for K in agreement with the exact results mentioned above for the limit $r/a \to 0$.

Turning to a linear polymer chain, we depict it by a trace of the path of a particle undergoing a series of random displacements; thus we seek an appropriate solution of the diffusion equation

$$\frac{\partial P_n(x,y,z)}{\partial n} = \frac{b^2}{6} \nabla^2 P_n(x,y,z) \tag{7}$$

where $P_n(x,y,z)\ dxdydz$ is the probability of finding the nth step of the random walk within a differential volume element $dxdydz$ located at a point x,y,z. The step length is normally distributed and its rms value is b. For the problem under discussion we suppose that the random flight begins at a point $(x',0,0)$ within a semi-infinite region $x > 0$ bounded by a plane at $x = 0$. Imposing the boundary condition that P_n vanish at $x = 0$, we can solve the differential equation to obtain:

$$P_n(x,y,z|x',0,0) = (6/\pi nb^2) \exp\{-(3/2nb^2)(y^2 + z^2)\} \times [\exp\{-(3/2nb^2)(x - x')^2\} - \exp\{-(3/2nb^2)(x + x')^2\}] \tag{8}$$

the probability that a random flight starting at $(x',0,0)$ arrives at the point (x,y,z) after n steps without encountering the boundary (*20*). Integration gives an error function,

$$P_{x'} \equiv \iiint P_n(x,y,z|x',0,0)\ dxdydz = \operatorname{erf}[(x'/2)(6/nb^2)^{1/2}] \tag{9}$$

where

$$\operatorname{erf} u = (2/\pi^{1/2}) \int_0^u e^{-t^2}\, dt \tag{10}$$

for the probability that a random flight beginning at a distance x' from the plane $x = 0$ and proceeding for n steps does not touch the boundary. Equivalently, $P_{x'}$ can be described as the fraction of all possible conformations of a random-flight polymer chain of n segments with one end at $x = x'$ in unbounded space that still remains available when an impenetrable boundary is placed at $x = 0$. The integral

$$x_c = \int_0^\infty (1 - P_{x'})\, dx' = 2(nb^2/6\pi)^{1/2} \tag{11}$$

represents an effective distance characterizing the depletion of polymer chains at equilibrium near the boundary. That is, in terms of the mean solute concentration (precisely, the concentration of chain ends), the cavity is equivalent to a volume in an unbounded space that is smaller than the real cavity by a layer of thickness x_c adjacent

to the wall. The distribution coefficient $K = K_1$ for the polymer species is then simply obtained by putting x_c in place of r in Eq. (6).

Just as in the case of rigid spheres, expressions for K_1 for flexible chain molecules derived in this way are correct limiting forms for molecules small compared to the dimensions of the cavity. Calculations not so restricted, of K_1 for random-flight chains in the slab, cylinder, and sphere cavities have been described elsewhere (*9*). The results are shown in Fig. 1 for comparison with the limiting behavior. It can be seen that the straight line representing the approximate K_1 for the slab crosses the curve for the exact relation at $K \approx 0.25$. There are similar intersections at much larger K for the cylinder and sphere relations, although this is not discernible on the scale of Fig. 1.

Since the mean-square radius of a random-flight linear chain of n segments is

$$R^2 = nb^2/6 \tag{12}$$

the quantity, $(nb^2/6a^2)^{1/2}$, where a is the radius of the cavity (half the separation of the planes in the slab model), is a convenient meas-

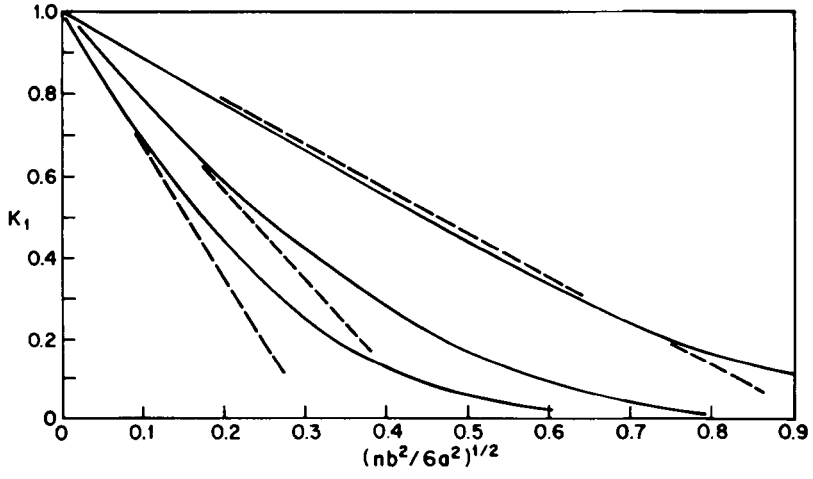

FIG. 1. Equilibrium constants for partitioning of linear random-flight polymer chains between a macroscopic solution phase and cavities of molecular dimensions. The solid curves (from top to bottom) are for slab-shaped, cylindrical, and spherical cavities. The abscissa is the ratio of the rms molecular radius to the cavity radius (half its thickness for the slab). The dashed lines are the corresponding limiting relations for the upper permeation limit, $K = 1$.

ure of the relative sizes of a polymer molecule and the cavity. For each pore geometry characterized by a single dimensional parameter a, it happens that K_1 will be a unique function of R/a. It can be seen from the plots, however, that these functions are quite different for the three cavity shapes. Although there is considerable interest in establishing a single combination of dimensional parameters to correlate (to some reasonable approximation) the equilibrium partitioning of any solute between a macroscopic solution phase and cavities of any geometry whatever (*10*), we shall pursue here only the more limited question of a comparison of the behavior of linear and branched polymer chains with respect to the same pore model. Thus the large absolute differences in K for a species in different types of pores are not our present concern.

Exact random-flight calculations would in general be very difficult for an arbitrary topology of chain branching. However, except for requiring lengthier computations, one model proves to offer no more real difficulty than do linear chains. This is what has been called the "regular star" model—a number of identical linear chain elements all joined by one end to a common branch point (*21*, *22*). The procedure described above for a linear chain near a plane is carried out for one of the branches: the origin of the chain is taken as the branch point, which is placed at x', and P_x is calculated as before for a chain of n steps. Now, however, f chains of the same length are generated in the same fashion from the same origin; and thus $(P_{x'})^f$ is the probability that a conformation of an f-fold star with the branch point at x' will not touch the boundary at $x = 0$. Finally, the distribution coefficient is obtained:

$$K_f = 1 - \sigma \int_0 [1 - P_x')^f] \, dx'$$
$$= 1 - 2\lambda\psi(nfb^2/6a^2)^{1/2} \qquad (13)^*$$

in which λ is a numerical factor dependent only on the cavity (1, 2, 3, for the slab, cylinder, and sphere, respectively) and ψ is a function of f that has been evaluated by numerical integration ($\psi = 1/\pi^{1/2}$ for linear chains). A few values of ψ are listed in Table 1. As the discussion has already implied, Eq. (13) is a correct limiting form for a polymer chain with average dimensions small compared to the cavity; it gives the first two terms of an exact series in powers of $(nfb^2/6a^2)^{1/2}$

* This relation was written incorrectly in Eq. (13) of Ref. *11*, with an extra factor $f^{1/2}$ in the last term.

TABLE 1

Effect of Chain Branching on Permeation Equilibrium for K_f near Unity

f	g	ψ	$\psi/g^{1/2}$	ν
1, 2	1.0000	0.5462	0.5642	
3	0.7778	0.5415	0.6140	0.163
4	0.6250	0.5178	0.6634	0.182
6	0.4444	0.4775	0.7163	0.206
8	0.3838	0.4458	0.7603	0.220
12	0.2361	0.3997	0.8226	0.239

and hence, the correct initial slope of K as a function of this variable for a given f. As in the case of linear chains, rather more complicated calculations give K for chains that are not small compared to the radius of the cavity (*11*).

CORRELATION OF RESULTS AND COMPARISON WITH EXPERIMENT

Equation (13) permits us to investigate the effect of chain branching on K_f. Qualitatively, it is apparent (and expected) that increasing f while keeping molecular weight fixed (keeping nf constant) increases permeation—because with increasing branching the molecule becomes more compact. Increasing n with f fixed decreases permeation since the molecular domain becomes larger; increasing f at fixed n also decreases permeation because the greater the number of branches, the greater is the chance that a conformation randomly generated from a given point inside a cavity will be interrupted by the walls of the cavity. The more important quantitative question is whether the dependence of K_f on branching can be correlated with any accessible measure of molecular size. The two most obvious choices are inadequate. If the total mass (or number of statistical segments) were the sole determinant, K_f would depend on nf/a^2 and ψ in Eq. (13) would be a constant; in fact, ψ decreases with increasing f. The rms molecular radius R is no more satisfactory as a correlating parameter. The effect of branching on mean molecular size is conventionally expressed by

$$g = (R_{br}/R_{lin})^2 \tag{14}$$

the ratio of mean-square radii of a branched chain and the analogous linear chain with the same mass. For random flight chains, R_{lin} is

given by Eq. (12) and the proportionality factor g has been obtained for a number of branched models, including the regular star (*21*). If K depended uniquely on R_{br}/a, the ratio $\psi/g^{1/2}$, according to Eq. (13) would have to be constant; actually it increases, as Table 1 shows.

These unsuccessful analyses represent attempts to express K_f first as a function of $(nfb^2/a^2)g^0$ and then of $(nfb^2/a^2)g$. That the deviations with f are in opposite directions suggests applicability of an intermediate power of g. In the last column of Table 1, we give values of ν defined by

$$\pi\psi^2 = g^{2\nu} \tag{15}$$

It is evident that when f is small, ν is not far from 1/5 or 1/6. Accepting the latter value, we can combine Eqs. (13) and (15), and propose

$$K_f = 1 - 2\lambda\pi^{-1/2}(nfb^2/6a^2)^{1/2}g^{1/6} \tag{16}$$

as a general relation for K_f for linear and branched chains provided K_f is not far from unity. Calculations for star molecules in slab, cylinder, and sphere cavities for $K < 1$ confirm in these more general cases a dependence on $R_{lin}g^{1/6}/a$, as Eq. (16) indicates. Plots of K_f versus $(nfb^2/6a^2)^{1/2}g^{1/6}$ at constant f superpose quite well, at least for $f \leq 8$. A slightly larger power of g improves the superposition for larger f (*11*). It is likely that these conclusions need not be restricted to star molecules since the ratio g correlates physical properties for a variety of branched chains.

Taking ν as precisely 1/6 was arbitrary; but this value, in conjunction with theoretical relations for the intrinsic viscosity, brings our results into conformity with a recent empirical determinaton. If the intrinsic viscosity for a linear chain species is given (*23*) by the product of a universal hydrodynamic constant Φ and the ratio R^3/M (M being the molecular weight) and if, according to an approximate theory of Zimm and Kilb (*24*), the intrinsic viscosity for star molecules is related to that for linear chains of the same mass by

$$[\eta]_{br} = [\eta]_{lin}g^{1/2} \tag{17}$$

it follows that

$$([\eta]_{br}M/\Phi)^{1/3} = (nfb^2/6)^{1/2}g^{1/6} \tag{18}$$

If the location of an elution peak in a chromatogram from a GPC column is governed by the quantity on the right-hand side of Eq.

(16), any polymer having the same value of the product $[\eta]M$ should elute at the same point *from the same column*. On empirical grounds, Benoit et al. (*25*) have proposed just this relation.

Our equilibrium calculations presuppose a theta-solvent system. The extension to polymer in a good solvent—in which the chain is expanded beyond random-flight size owing to potentials of mean force $U_0\{q\}$ that depend on conformation—can be done to an adequate approximation, at the price of loss of elegance; however, there is some reason to believe that results for a random flight modified simply by use of the mean-square size of the real chain will not be seriously in error (*26*). Probably a more important shortcoming of the theory is the total neglect of adsorption of polymer on the column packing. It is very likely that this is a perturbing influence in many situations of practical interest.

It is important to note that the ideas proposed here are susceptible to experimental tests apart from verification of the predicted universality of $[\eta]M$ as a column calibration parameter. In the unlikely event that the real pore structure were sufficiently simply and precisely known, K for a polymer species of known molecular dimensions could be calculated *a priori* and compared with the apparent value deduced from elution measurements with the aid of Eq. (4). The column material coming closest to such idealized requirements is a special porous glass developed by Haller (*27*, *28*), which has been found by electron microscopy and mercury intrusion measurements to have pores of remarkably uniform cross section. Values of K determined from elution studies (*29*) of a series of nearly monodisperse linear polystyrenes from two columns of such glass with different pore sizes agree well with the theoretical relation for K versus R/a for the slab model (*9*). However, since the experimental estimates of a (by mercury intrusion) (*27*) may be subject to systematic error, the results can be taken as indicating no more than that the theory gives results of the right order.

Finally, it is important to remember that the validity of interpreting K in Eq. (4) as an equilibrium constant can be studied without regard to a particular model. The value of K can be obtained directly in a static experiment, by equilibrating an aliquot of polymer solution with the porous gel and determining the change in concentration in the supernatant liquid, provided that a calibration experiment with a solute species too large to penetrate the pores is also done. The equilibrium data can then be compared with results from

column elution. In a recent study of this kind, Yau, Malone, and Fleming (*30, 31*) found agreement between static and chromatographic experiments for elution of polystyrene solutions from porous glass. With the more familiar cross-linked polystyrene gel as a column packing, deviations from equilibrium behavior were found—elution volumes exhibiting a dependence on flow rate that became more pronounced with increasing molecular weight.

Acknowledgment

This study was aided by the Air Force Materials Laboratory.

REFERENCES

1. W. W. Yau and C. P. Malone, *J. Polym. Sci., Part B,* **5,** 663 (1967).
2. G. K. Ackers, *Biochemistry,* **3,** 723 (1964).
3. K. O. Pedersen, *Arch. Biochem. Biophys., Suppl.* **1,** 157 (1962).
4. C. Gutmann and E. A. DiMarzio, *J. Polym. Sci., Part B,* **7,** 267 (1969).
5. F. H. Verhoff and N. D. Sylvester, *J. Macromol. Sci.—Chem.,* **A4,** 979 (1970).
6. J. Porath, *Pure Appl. Chem.,* **6,** 233 (1963).
7. T. C. Laurent and J. Killander, *J. Chromatogr.,* **121,** 317 (1964).
8. P. G. Squire, *Arch. Biochem. Biophys.,* **107,** 471 (1964).
9. E. F. Casassa, *J. Polym. Sci., Part B,* **5,** 773 (1967).
10. J. C. Giddings, E. Kucera, C. P. Russell, and M. N. Myers, *J. Phys. Chem.,* **72,** 4397 (1968).
11. E. F. Casassa and Y. Tagami, *Macromolecules,* **2,** 14 (1969).
12. R. L. Pecsok and D. Saunders, *Separ. Sci.,* **1,** 613 (1966).
13. J. C. Giddings and H. Eyring, *J. Phys. Chem.,* **59,** 416 (1955).
14. J. C. Giddings, *J. Chem. Phys.,* **26,** 169 (1957).
15. D. A. McQuarrie, *J. Chem. Phys.,* **38,** 437 (1963).
16. J. B. Carmichael, *J. Polym. Sci., Part A-2,* **6,** 517 (1968).
17. T. L. Hill, *Statistical Mechanics,* McGraw-Hill, New York, 1956, Chapter 6.
18. P. J. Flory, *Principles of Polymer Chemistry,* Cornell Univ. Press, Ithaca, N. Y., 1953, Chapter 12.
19. A. G. Ogston, *Trans. Faraday Soc.,* **54,** 1754 (1958).
20. H. S. Carslaw and J. C. Jaeger, *Conduction of Heat in Solids,* 2nd ed., Oxford Univ. Press, London, 1959. Chapter 10.
21. B. H. Zimm and W. H. Stockmayer, *J. Chem. Phys.,* **17,** 1301 (1949).
22. T. A. Orofino, *Polymer,* **2,** 295, 305 (1961).
23. P. J. Flory, Ref. 18, Chapter 14.
24. B. H. Zimm and R. W. Kilb, *J. Polym. Sci.,* **37,** 19 (1959).
25. H. Benoit, Z. Grubisic, P. Rempp, D. Decker, and J. G. Zilliox, *J. Chem. Phys.,* **63,** 1507 (1966).
26. D. J. Meier, *J. Phys. Chem.,* **71,** 1861 (1967).
27. W. Haller, *Nature,* **206,** 693 (1965).
28. W. Haller, *J. Chem. Phys.,* **42,** 686 (1965).

29. J. C. Moore and M. C. Arrington, International Symposium on Macromolecular Chemistry, Tokyo and Kyoto, 1966. Preprints VI-107.
30. W. W. Yau, C. P. Malone, and S. W. Fleming, *J. Polym. Sci., Part B,* **6,** 803 (1968).
31. W. W. Yau, *J. Polym. Sci., Part A-2,* **7,** 483 (1969).

Received by editor May 11, 1970