correct, the wrong assignment in Figure 1 of ref 29b is probably a typographical error.

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(35) As pointed out in ref 60 of paper I,[3] there are errors in the values of P_α and P_β in Table II of ref 29a, and hence in Table I of ref 29b, because of a mathematical error. However, in the illustrative examples in the present paper, we retain the numerical values of Chou and Fasman to compute $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$.

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Comments on Exclusion of Polymer Chains from Small Pores and Its Relation to Gel Permeation Chromatography

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Received August 15, 1975

ABSTRACT: Some criticisms of our theoretical treatment of the partial exclusion of flexible-chain polymers in solution from cavities of macromolecular size and its application to gel permeation chromatography are examined. In other discussion, it is confirmed by simple reasoning that the identification, explicit or implicit in various studies, of the mean projection of a polymer molecule onto a line as a characteristic dimension governing the extent of permeation of simple pores does not depend on specific molecular models. Our previous calculation of permeation by certain random-flight branched-chain species is shown to lead, incidentally, to the mean projection for these structures. From relations between the mean projection and the hydrodynamic volume of a molecule, it appears that the product of intrinsic viscosity and molecular weight is not a common calibration factor for elution of all molecular species from a gel chromatographic column, but theory and experience do support the validity of this correlation among solutes with similar molecular architecture.

Although it has been a subject of controversy over the last decade, it now seems generally agreed that peak separation in gel permeation chromatography (or exclusion chromatography, to use a better but less accepted term) is governed primarily by the equilibrium distribution of solute species between a macroscopic solution phase (the mobile phase in chromatography) and solution within pores of macromolecular size.[1–6] In advocating this point of view on chromatography of flexible-chain polymers, we calculated distribution coefficients for random flight chains in cavities of simple geometrical form with rigid impermeable, but otherwise noninteractive, walls.[1,3–5] For these idealized models, the problem is to obtain appropriate solutions of the equation

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

where $P_n(\mathbf{r})$ is the probability density for finding the nth step of a random flight with root-mean-square length b at a point designated by a vector $\mathbf{r}$ drawn from the coordinate origin. In an infinite medium the familiar Gaussian form for $P_n(\mathbf{r})$ is obtained, but by imposing the boundary condition that all $P_n(\mathbf{r})$ vanish when $\mathbf{r}$ is on the surface S defining the walls of the cavity, the equation can also be solved for a random flight within a cavity of sufficiently simple geometry. The probability $P_n(\mathbf{r}|\mathbf{r}')d\mathbf{r}$, thus obtained, that a chain of n steps beginning at $\mathbf{r}'$ will terminate in the volume element $d\mathbf{r}$ without ever intersecting S is averaged over all $\mathbf{r}$ inside S to obtain the probability that a chain beginning at $\mathbf{r}'$ will not touch the boundary, and a final integration over $\mathbf{r}'$ gives the permeation (or distribution) coefficient K, the fraction of all unrestricted chain conformations beginning inside S that is still allowed in the presence of the impenetrable barrier or, equivalently, the ratio of polymer concentrations in solutions inside and outside cavities. Certain artifices may simplify calculation (e.g., imagining random flights to be generated simultaneously throughout the volume of the cavity), but results are equivalent to those obtained by enumerating conformations of a single chain.

Since our theoretical work was published, several authors have questioned some aspects of it or its application, and/or have suggested amplifications of it. Here we comment on three of these studies, and, at the end, discuss the relation between a characteristic molecular dimension governing the exclusion phenomenon and the question of a "universal" calibration in gel chromatography.

I. Confrontation of Theory and Experiment. Characterization of Pore Size and Shape

If K is identified with the effective fraction of pore volume available to solute in a chromatographic column, the theoretical calculations can be compared with elution data. Yau and Malone[6] have addressed themselves to the comparison of theory and experiment that we made in ref 1. Using the same elution and pore size data, together with newer results from their laboratory, they show how our analysis should be improved.

In ref 1, we plotted theoretical values of K against a dimensionless size parameter R/a, the ratio of the root-mean-square molecular radius of gyration, $R = (nb^2/6)^{1/2}$, of a linear random-flight chain to the pore size a (half the thickness of the slab-shaped cavity, or the radius of the cylinder or sphere), and compared the curves with the experimental dependence of K deduced from column elution data[7] for a series of narrow distribution polymers on columns of porous glass having especially uniform pore radii, as determined by mercury porosimetry. The polymer chain dimensions were known independently, and we cavalierly took the ratio of experimental molecule and pore sizes as interchangeable with R/a for the theoretical models. However, as Yau and Malone point out, the pore radii from intrusion porosimetry are those of averaged equivalent circular cross-sections of pores, and geometry indicates that these effective radii should be identified as $2a$, a, and $2a/3$ for the idealized uniform slab, cylinder, and sphere cavities, respectively. We take these relations as *defining* the effective radii, which we here designate by a_p.[8] It is notable that the ratios a/a_p stand in the porportions 1:2:3, i.e., as the surface-to-volume ratios of slab, cylinder, and sphere, respectively. It also turns out that at the limit $K = 1$ for complete permeation (i.e., for small molecules in large pores) our theoretical derivation yields[3]

$$\lim_{K=1}\,[\mathrm{d}K/\mathrm{d}(R/a)] = -2\lambda/\pi^{1/2} \qquad (2)$$

with $\lambda = 1, 2, 3$, in order, for slab, cylinder, and sphere. This circumstance means that the theoretical functions for K for the several pore models are superimposed, to the extent of a common initial slope, when plotted against $\lambda R/a$, or against any quantity, like R/a_p, proportional to it.

In Figure 1, we plot theoretical K's against R/a_p together with some experimental data, with the pore sizes measured by porosimetry (Yau and Malone use a logarithmic scale for the reduced size variable but our linear coordinates are better for exhibiting behavior at small R/a.) Comparing Figure 1 here with Figure 1 of ref 1, we see that the agreement previously apparent between experiment and the slab model has disappeared and permeation appears greater for all the pore shapes than is predicted by theory. The introduction of a better measure of pore size corresponding to experiment does not, however, alter the earlier comparison most significant physically, that with the cylindrical pore model. The large quantitative descrepancy between theory and experiment finds a plausible explanation in the "ink-bottle" effect in mercury porosimetry; in real systems large voids with constricted openings are counted as having the cross-section of the entrance and, consequently, the measured pore size is too small. Combining porosimetry and gas adsorption measurements of surface area on a porous glass, Yau and Malone find a corrected pore size about twice the apparent value from intrusion measurements. A correction of this magnitude is evidently the adjustment needed to move experimental points in Figure 1 to the left into reasonable juxtaposition with the theoretical curves.

Despite the gratifying nature of the final agreement, the extreme idealization of the pore models and the difficulties of interpreting pore size measurements suggest skepticism of *a priori* quantitative predictions of K from theory. Similarly, despite the lack of complete superposition of the lower three curves in Figure 1, it seems likely that attempts to assess pore shape solely from the experimental dependence of K on R would be unrewarding.

Yau and Malone represent our original comparison of theory with experiment in ref 1, our introduction of λ in ref 3, and their work reported in ref 6 as constituting successive identifications of a characteristic dimension for permeation of pores in gel chromatography, i.e., a, a/λ, and $2a/\lambda \equiv a_p$, respectively. There is nothing logically inadmissible in this description, but it seems to us that their argument obscures the distinction between the theory of gel chromatography and the separate matter of pore size measurement. In ref 3, our introduction of the shape factor λ to harmonize the initial slopes of the theoretical curves was justified by an appeal to the theory of the permeation coefficient; we did not propose it as a means of rationalizing theory with experiment. Our theoretical development is entirely consistent in terms of the unambiguously defined pore size a and, contrary to an inference that might be drawn from Table I of ref 6, there is no disjuncture between ref 1 and 3. Yau and Malone combine the theories of chromatography and of porosimetry to identify a_p as "the fundamental permeability parameter for GPC" because, in the context of the idealized models for pores and porosimetry, this effective pore dimension would harmonize theory and experiment for K as a function of molecular size R. On this point, we remark that if pore radii were measured by a method that involved some other averaging of physical dimensions, the "fundamental parameter" deduced this way would be a different one. Consequently, with no intent of depreciating the happy circumstance that R/a_p is an appropriate variable to use in correlating theory with available experiments (or the obvious value in Yau and Malone so demonstrating), we prefer to regard the relation between a and a_p as one determined by a particular model for pore-size determination and having nothing to do conceptually with gel chromatography per se.

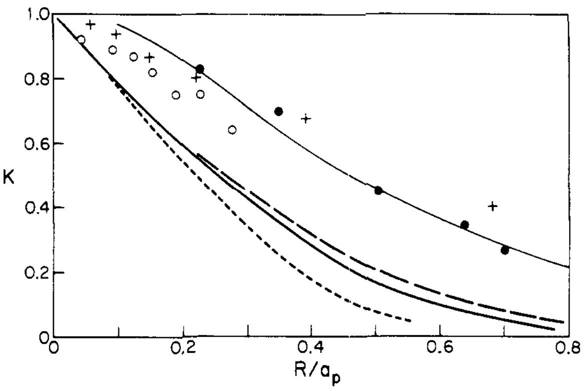

Figure 1. Dependence of the distribution coefficient on the molecular size/pore size ratio R/a_p for linear flexible-chain polymers. The lower curves are our theoretical results[1] for random flights in slab-shaped (short dashes), cylindrical (thick solid curve), and spherical (long dashes) cavities. The uppermost curve, transcribed from ref 9, is the theoretical function of Vilenchik et al. for cylindrical pores. The experimental points are determined by column elution data for polystyrene on porous glass and apparent pore radii from mercury intrusion data from Moore and Arrington[7] (open and filled circles) for a series of polymers with two glasses of different porosity and from Yau and Malone[6] (crosses).

II. Another Calculation of Permeation Coefficients

More recently, Vilenchik et al.[9] have carried out a new calculation of K for linear random-flight chains in slab, cylinder, and sphere cavities, and report results far different from those in ref 1. Their work is surely incorrect, but it cannot be fully evaluated because they give no analytical solutions (eq 1 is said to have been solved numerically "by an implicit difference scheme") and display results only in graphical form. The most informative plots are those in Figure 3b of their paper, which exhibits K vs. R/a, although the abscissa is mislabeled as log (R/a). The Vilenchik function for cylindrical cavities is reproduced with our theoretical K's in Figure 1. As in this example, their values of K are always larger than ours, and, in contradiction with eq 2, the curves appear to approach $K = 1$ with zero slope. Finite negative initial slopes are in fact dictated by considerations of general character without reference to random flights.[2-4] When the permeating particle is small in comparison with the pore dimension a, the effectively accessible volume fraction of the cavity is determined by two factors: its surface per unit volume σ and a characteristic dimension x_c of the permeating species. Together, these give the volume of a layer adjacent to the cavity walls that must be subtracted from the actual volume. Then K is just the ratio of the effective volume to the total pore volume

$$K = 1 - \sigma x_c \tag{3}$$

and, accordingly, $\partial K/\partial x_c$ does not vanish when x_c becomes small, as eq 2 shows for a special case.

Although the boundary conditions assumed by Vilenchik et al. in solving eq 1 appear to be equivalent to ours,[10] their plots of K vs. R/a are reminiscent of the sigmoid curves obtained by constraining polymer chains in a cavity to have one end at the center.[11] Vilenchik et al. attribute to us an "inappropriate" analogy with the theory of heat conduction" and add that we "should have considered the cooling of a body containing initially a point heat source which could be placed with equal probabilities at different points in the body, rather than the cooling of a uniformly heated body". However, both approaches, pursued consistently, lead to the same results, and the recommendation of Vilenchik et al. is actually followed in ref 3.

Vilenchik et al. cite experiments as support for their calculations. Agreement is also exhibited with the data points plotted in Figure 1, but this would be invalidated by the "ink-bottle" correction suggested by Yau and Malone.

III. A Characteristic Molecular Dimension for Exclusion from Cavities

In ref 3, we calculated x_c for a random-flight chain by solving eq 1 for the probability P_x that a chain beginning at a distance x from an infinite plane does not intersect the plane and then averaging over all x the probability that it *does* intersect the plane: i.e.

$$x_c = \int_0^\infty (1 - P_x)\mathrm{d}x \tag{4}$$

Now, van Kreveld[12] has pointed out that our result, $x_c = 2\pi^{-1/2}R$, is just half the mean projection of the chain onto a line, a quantity calculated long ago by Kuhn[13] and by Weidmann et al.[14] In the remaining discussion, we explore some general implications that emerge from van Kreveld's observation in conjunction with earlier work.

In considering permeation of simple pores by rigid particles, Giddings et al.[2] also used the mean molecular projection (or as they termed it, "the mean external diameter") as a dimension to characterize exclusion. For the present purpose, the important point is that they also found x_c to be half the mean projection of the molecule. The identification is trivial for a spherical molecule; its radius, which is also half its projection, is the minimum distance between its center of mass and the wall of the cavity. Enumeration of configurations of a thin rod of length l near a plane wall[2,15] yields $x_c = l/4$. If the rod is imagined with one end fixed at the origin of polar coordinates, possible orientations are described by points on the surface of a sphere of radius l and the projection on the polar axis is $l \cos \theta$. Averaging over all orientations gives

$$\int\int l^3 \cos\theta \sin\theta \,\mathrm{d}\theta\,\mathrm{d}\phi / \int\int l^2 \sin\theta\,\mathrm{d}\theta\,\mathrm{d}\phi = l/2 \tag{5}$$

or twice x_c. For a "broken" rod formed by connecting two rods of length $l/2$ by a universal joint, slightly more complicated calculation gives $5l/12$ for the projection, which again comes out to be $2x_c$.[15]

These results make it evident that the identification of the exclusion distance x_c as half the mean molecular projection is independent of molecular geometry. That this is so is confirmed by a simple argument. Letting an instantaneous projection of the molecule on a line be X, we have, by definition of the mean, $\bar{X}$,

$$\frac{\bar{X}}{2} = \int_0^\infty \frac{X}{2} W(X)\,\mathrm{d}X \tag{6}$$

where $W(X)\,\mathrm{d}X$ is the probability of finding X in the range $\mathrm{d}X$. From symmetry, it is obvious that the population comprising all conformations with a given value of X perpendicular to a plane permits the center of mass of the molecule to approach the wall to a distance $X/2$, on the average. Then, eq 6 expresses the final averaging over all values of X, and $\bar{X}/2$ is the thickness of the exclusion layer at the wall.[12]

With this entirely general connection between x_c and $\bar{X}$ established, we can obviously assert that calculation of the former provides a route to the latter. In the case-of random-flight chains, at least, the calculation of x_c proves to be simpler than the rather intricate derivations of $\bar{X}$ described by Kuhn[13] and Weidmann et al.[14,17] We note that our calculation in ref 3 of permeation coefficients for star-shaped branched chains, (f identical linear chains, each of n segments, attached by one end to a common junction) incidentally affords values of $\bar{X}$ for these models. The result is

$$\bar{X} = 4(nfb^2/6)^{1/2}\psi \tag{7}$$

with

$$\psi = \left(\frac{f}{\pi}\right)^{1/2} \int_0^\infty (\operatorname{erf} t^{1/2})^{f-1} e^{-t}\,\mathrm{d}t \tag{8}$$

where erf denotes the error function. The definite integral has been evaluated numerically.[3,18] If f is not too large, a fair approximation is

$$\bar{X} \approx 4(nfb^2/6\pi)^{1/2}[(3f - 2)/f^2]^{1/6} \tag{9}$$

The quantity within brackets is the ratio of mean square radii of branched and linear chains of the same mass.[19]

Unequivocal identification of $\bar{X}$ via eq 4 and 6 as a characteristic molecular dimension governing exclusion from the neighborhood of an impenetrable barrier for any molecular model prompts a further assessment of the popular empirical correlation of elution from a gel chromatographic column with the product $[\eta]M$ of intrinsic viscosity and molecular weight. Since $[\eta]M$ is proportional to the effective hydrodynamic volume of the molecule, a common dependence of elution volumes of all species from a given column on $[\eta]M$ would imply, as has been proposed, that the hydrodynamic radius constitutes a "universal" calibration parameter in gel chromatography.[20] Were this so, there would have to exist a unique quantitative relation, independent of molecular geometry, between the hydrodynamic radius and $\bar{X}$. For hard spheres, equating $\bar{X}$ to the actual diameter, we have from the Einstein relation

$$[\eta]M = (5\pi N_A/12)\bar{X}^3 = 7.88 \times 10^{23}\bar{X}^3 \tag{10}$$

in cgs units with N_A denoting Avogadro's number. For linear random-flight nondraining chains, the corresponding relation is[21]

$$[\eta]M = \Phi(\pi^{3/2}/64)\bar{X}^3 = 3.43 \times 10^{23}\bar{X}^3 \tag{11}$$

with the constant Φ taken[22] as 39.4×10^{23}. An approximate treatment of the intrinsic viscosity of branched chains by Zimm and Kilb[23] predicts that eq 11 should also hold for star molecules with $\bar{X}$ given by eq 7. Finally, for impenetrable cylindrical rods of large axial ratio p and molar volume V_0, a theory of Simha[24] leads to a form

$$[\eta] \approx V_0 p^2 \Omega(p)/5M \tag{12}$$

where

$$\Omega = [3 \ln (2p) - 5.4]^{-1} + [\ln (2p) - 0.8]^{-1} \tag{13}$$

is a slowly decreasing function of p (0.59 for $p = 15$, and 0.27 for $p = 200$). Eliminating the volume V_0 by introducing $\bar{X} \approx l/2$, we obtain

$$[\eta]M \approx (2\pi N_A/5)\Omega\bar{X}^3 = 7.57 \times 10^{23}\Omega\bar{X}^3 \tag{14}$$

Despite uncertainties in all but perhaps the first of these three expressions for $[\eta]M$, the quantitative differences among them and appearance of a new functional dependence in eq 13 suggest that $[\eta]M$ is not a truly universal elution parameter for gel chromatography, but these relations also suggest, in agreement with experience, that the correlation should be a good one for species of similar type, e.g., for flexible chains both linear and with a limited amount of long-chain branching, or for rodlike polymers of similar molecular cross section in a restricted molecular weight range. We note in particular that eq 11 and 14 coincide for $p = 33$. This indicates a range in which a common $[\eta]M$ dependence should correlate elution of both freely coiling and rodlike molecules. Such agreement has been seen experimentally.[25] The theoretical evidence thus points to a significant difference in the $[\eta]M$ calibration between compact spheroidal particles and more extended structures, with no great sensitivity to molecular flexibility in the latter class.

It must be remembered that these theoretical correlations are worked out only for the limit of large K where the very simple form of eq 3 holds. While they may apply as well over a useful range of permeation, the situation for small K, with particles comparable in size with the cavities, will be more complicated.[2,3,15]

Acknowledgment. This work was aided by the Air Force Nonmetallic Materials Division, Polymer Branch, under Contract No. F33615 70 01058.

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(16) That an averaged molecular projection should be an appropriate quantity to characterize gel chromatographic separation of coiling polymers was perhaps first suggested by Moore and Arrington.[7]

(17) The treatments of $\bar{X}$ in ref 13 and 14 are more fundamental than our calculation in the sense that they give the distribution $W(X)$ as well as the mean.

(18) Since it is the primary source for much of the material discussed here, it seems fitting to record some errors of transcription found in ref 3. An extraneous factor $f^{1/2}$ must be deleted from eq 13 to make it read

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

With spurious parentheses removed, the related eq A12 can be written

$$(K_f)_{\mathrm{slab}} = 1 - 2u\left\{\frac{2f}{\sqrt{\pi}}\int_0^\infty \xi(\operatorname{erf}\xi)^{f-1}\exp(-\xi^2)\,d\xi\right\} + O(u^2)$$

More obvious slips occur in Table I, where the caption should read "high permeation" not "small permeation", and in eq 19 where the left-hand side should be $V_e - V_0$. Reference 18e should read "eq 3.3(9)" and ref 18h should read "eq 2.4(4)". These mistakes in no way affect conclusions or numerical results.

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