Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

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F. H. Verhoff[a]; N. D. Sylvester[a]
[a] Department of Chemical Engineering, University of Notre Dame, Notre Dame, Indiana

To cite this Article Verhoff, F. H. and Sylvester, N. D.(1970) 'Hydrodynamic Fractionation of Macromolecules. I. A Simple Theory', Journal of Macromolecular Science, Part A, 4: 4, 979 — 1001
To link to this Article: DOI: 10.1080/00222337008060992
URL: http://dx.doi.org/10.1080/00222337008060992

J. MACROMOL. SCI.–CHEM., A4(4), pp. 979-1001, July, 1970

Hydrodynamic Fractionation of Macromolecules. I. A Simple Theory

F. H. VERHOFF and N. D. SYLVESTER

Department of Chemical Engineering
University of Notre Dame
Notre Dame, Indiana 46556

SUMMARY

A new mechanism for separation in gel permeation chromatography (GPC) or gel filtration has been developed based upon the postulate that flow occurs through the gel phase. On the basis of the hydrodynamic nature of the separation mechanism, the new name Hydrodynamic Fractionation is proposed. A theory has been formulated for the case of equal pore size in the gel phase and equal sized spaces between the beads. This theory predicts the elution volume vs molecular weight curve. The flow-rate dependence of the separation peaks is investigated.

The theoretically predicted relationship between molecular weight and elution volume compares very well with the gel permeation experiments using glass beads as packing probably because these packings correspond most closely to the assumptions in the theory. In the cross-linked polymer packings where a large distribution of pore sizes exists the theory does not fit well; however, it does predict the general shape of the curve. The flow-rate dependence of the separation peaks is experimentally the same as qualitatively predicted. Also the experimental findings of equilibrium experiments were correlated with the corresponding GPC data using the present theory.

INTRODUCTION

Gel permeation chromatography (GPC) or gel filtration, an important technique for the separation of synthetic and biological polymers according to size, has been experimentally investigated by numerous workers in the recent past; see, for example, the reviews by Altgelt and Moore [1], Johnson, Porter, and Cantow [2], Determann [3], and others [4-7].

Studies of sharp molecular weight fractions of linear polymers in GPC [2, 6] allowed the development of an empirical linear relationship between the peak of the elution volume curve and the logarithm of the molecular weight over some range. In particular it has been found that the hydrodynamic volume is the factor determining the elution volume associated with a particular molecule [8-14]. Since the elution volume peaks of the sharp fractions have a significant spread, various investigators [15-20] have focused on methods of correcting chromatograms for this variance. Also some experimenters have looked at other influencing factors including that of viscosity difference between that of the eluant and the sample [21-24], the flow rate [8, 22, 24-27], sample size [21-23, 28], temperature [8, 24], and the solvent [24]. Although most GPC studies have employed either cross-linked polystyrene or dextran (Sephadex) gels, the porous glass bead packings [29-36] are better suited for fundamental studies of the mechanism of separation because their pore size distributions are narrower and can be measured by conventional means (e.g., mercury porosimeter measurements, gas desorption isotherms, and electron micrographic measurements [30, 32, 34]). The general goal of these experiments has been to determine feasible operating conditions for GPC and to calibrate the chromatograph such that the molecular weight distribution of unknown polymers can be calculated from their chromatograms. Thus far these experimental results have been treated empirically because none of the proposed theories for the separation process have been in complete agreement with the experiments.

The theories of gel permeation chromatography can be conveniently divided according to the physical process assumed to accomplish the separation, i.e., volume exclusion, restricted diffusion, or entrapment. The volume exclusion theory [37-42] assumes that cavities of various sizes in the size range of the polymer molecules exist in the stationary phase of the bed. As the molecules move through the bed in the mobile phase, they are assumed to be in equilibrium with those in the stationary phase. Since each polymer of a different size "sees" a different volume in the stationary phase, it will elute at different times. The larger molecules flow through a smaller volume and elute first. This theory has gained the most attention because

certain qualitative and quantitative predictions derived from it seem to agree with experiment.

The critical assumption in this theory appears to be the assumed equilibrium between the stationary and moving phase. This assumption can be checked for the case of the glass beads. For a bead of radius 100 μ located in a GPC column, the time required for this bead to reach 90% saturation for a step function change in the concentration of a small molecular species around it is about 3 sec [45]. (This assumes that the mass transfer Biot number [43-45] around the particle is infinite; it really is probably in the range of 1, which would make the time larger). For equilibrium to exist, the phase outside the bead should not have moved much more than the length of the bead. This gives a velocity through the bed of 6.6×10^{-3} cm/sec; the time to pass through a 66 cm bed, retention time, would be 10^4 sec or about 3 hr. It should be noted that this low estimate is far greater than the retention times used in similar glass bead GPC columns [32, 34-36]. For these columns the assumed equilibrium between the flowing phase and the stationary phase simply does not exist.

The restricted diffusion theory [32, 36, 42, 46] predicts that the variation in diffusion rates of the different size polymer molecules accounts for the separation. As the polymer passes through the bed the faster diffusing species (the smaller molecules) will be able to penetrate more of the void space in the stationary phase and hence elute at a later time. However, in such a separation process, the peak elution volume would be strongly dependent on flow rate; this is not found from experiment [8, 22, 24-27]. Haller [32] suggests a storage effect but then seems to disprove it himself.

The entrapment theory [47-51] postulates that a molecule moves through the bed by a series of entrapments into the stationary phase and elution into the mobile phase. Both entrapment and elution are assumed to be Poisson processes although no physical reason for this choice is given. Also, the physical phenomenon of entrapment is not described. The resultant volume elution curves are then calculated for a bed of all the same size pores and for one with a distribution of sizes [51]. If two adjustable parameters are properly but arbitrarily chosen, quantitative agreement between experiment and theory is found for certain ranges of molecular weight. The author [51] points out that although his model fits the data with two adjustable parameters, it may not be correct.

The inadequacies of the above theories indicate that the mechanism of separation in GPC is not understood and that most of the proposed mechanisms are incorrect or incomplete. The purpose of this paper is to propose a new mechanism of separation, to develop a theory from this mechanism, and to

compare theoretically predicted results with those taken from experiment. The theory developed herein will be specifically for the glass beads although its generality for gel-type packings should be apparent.

The nomenclature used in connection with the separation process described is complex and confusing and certainly will not be readily resolved. The various names that have been used are gel filtration [38], exclusion chromatography [52], restricted diffusion chromatography [53], molecular-sieve filtration [54], molecular-sieve chromatography [55], and gel permeation chromatography [25]. The word chromatography in these terms refers to the manipulative steps involved in the experimental procedure rather than to the operative mechanism of separation. Classically, chromatography refers to a separation mechanism due to differences in the interaction between various solutes and the surface of the chromatographic medium. In GPC, however, adsorption takes little or no part in the separation [31]. Diffusion has also been shown to be of minor importance [32]. Since the separation is not chromatographic, the simplest and first used term, gel filtration, might seem most appropriate, although the term gel permeation chromatography is most popular with investigators of synthetic polymers [2] and an excellent argument for the choice of gel chromatography has been made [56]. However, due to the hydrodynamic nature of the new mechanism proposed here for the fractionation of macromolecular species of different size, the name Hydrodynamic Fractionation is proposed. Although this is a new name and may lead to further confusion, it is felt necessary, by the authors, inasmuch as it most accurately describes the separation phenomena.

PHYSICAL MECHANISM OF SEPARATION

All previous theories of GPC have assumed that the eluant is stationary in the gel phase (glass beads or cross-linked gel), i.e., that the velocity of the eluant in the direction of flow is zero. The polymer molecules are assumed to diffuse into and out of this stationary eluant as they pass through the bed. Hallers experimental data [32] on the diffusion into the glass beads shows a rapid uptake of small and intermediate size molecules which appears to contradict this assumption although a critical test is not possible because of insufficient information.

From the description of how the porous glass beads are formed [30, 31] it appears that the pores are interconnected and that flow through the beads is possible. Similarly it appears that pores exist in the polymer

packings. Thus if one of these beads was located in a bed, the fluid flow patterns in the vicinity of a bead may be as shown in Fig. 1. This flow through the bead occurs because of the pressure gradient along the surface of the bead.

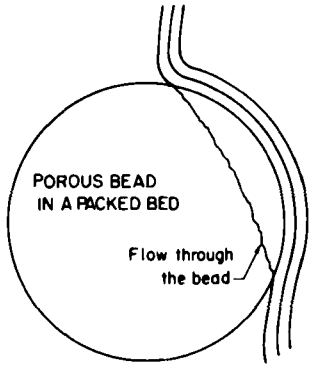

Fig. 1. Fluid flow patterns near porous beads in a packed bed.

To demonstrate the flow phenomena in such a bed, two molecules are considered as they approach a bead in the fluid outside. Suppose that because of its size the smaller molecule enters the pore but the larger one remains outside. The larger molecule will reach the end of the particle sooner than the small one because the fluid velocity outside the bead is much faster than inside. Hence at this point the larger molecule will be separated from the smaller one and would elute from the bed first.

Although not of extreme importance, calculations based on some average pore sizes and lengths indicate that the time required for a small molecule to flow through a bead is much less than that of the molecule flowing through the whole bed outside the beads. Thus molecules which can penetrate the beads will pass through a series of glass beads. As in previous theories, various effects such as adsorption while proceeding through the bead are neglected. Since the smaller molecules more easily enter the porous beads (and since the flow rate is much slower in the pores than outside) they will be eluted after the larger molecules which enter the pores with greater difficulty or not at all.

In summary, the mechanism of GPC separation proposed herein is hydrodynamic, i.e., the molecules are separated because of the flow patterns they follow through the bed. The small molecules flowing into the pores of the beads will be delayed and hence elute at a later time than those that enter

the pores with some difficulty or not at all. Since the separation process is hydrodynamic, the elution volume will depend upon a "hydrodynamic" molecular size as has been found from experiment [8-14].

Diffusion itself will play only a minor role in this GPC separation theory except in the case of extremely low flow rates. Its main effect will be increased dispersion as would be expected in a packed bed [57-60]. The molecules that diffuse into the holes from outside are countered by the outward diffusion of the molecules carried in by flow. For very small bead pores the flow through the bead is very slow and if a molecule could stay trapped in this pore it would elute from the bed at a very long retention time. Diffusion assures that it won't stay trapped more than from 3 to about 100 sec. This phenomena is similar to the fact that if a molecule would stay trapped in a low velocity streamline near a particle in flow through a packed bed, such a molecule would elute from the bed at very long retention times. However, diffusion into the higher velocity streamlines assures that this doesn't happen.

PREDICTION OF ELUTION VOLUMES

As a molecule flows through a porous glass bed, it passes through a series of different pore sizes. For simplicity, we will assume two pore sizes available; the interstitial pore size between the glass beads, r_2, and the pore size in the glass beads, r_1. Further it is assumed that all molecules pass through exactly N holes of the same length; the number of each size hole comprising the total N is a random variable.

First, we will develop an expression for the average elution volume of a molecule much smaller than the small pores. At each one of the N steps the small molecule can enter either a large pore or a small pore. Its probability of entering a small pore, P_1, is equal to the solvent flow rate through the small holes divided by the total flow rate, i.e.

$$P_1 = \frac{u_1 \frac{V_1}{L}}{Q} \tag{1}$$

where V_1 is the volume of small pores in the bed, u_1 is the velocity through the small pores, L is the length of the bed, and Q is the total flow rate through the bed. Similarly, the probability that it will enter a large pore is

$$P_2 = \frac{u_2 \frac{V_2}{L}}{Q} \tag{2}$$

From this it is evident that

$$P_1 + P_2 = 1$$

and hence

$$Q = u_1 \frac{V_1}{L} + u_2 \frac{V_2}{L} \tag{3}$$

The time spent in each one of the pores will then be $(L/N)/u_i$, and the total time spent in the bed is

$$t_S = \sum_{i=1}^{N} (L/N)/u_i \tag{4}$$

The average value of the total time, assuming that the probability of entering a hole is independent of previous holes, is

$$\bar{t}_S = E(t_S) = \frac{L}{N} \sum_{i=1}^{N} E(1/u_i) = LE(1/u_i) \tag{5}$$

where

$$E(1/u_i) = P_1 \frac{1}{u_1} + P_2 \frac{1}{u_2}$$

Substituting for P_1 and P_2 from Eqs. (1) and (2) gives

$$E(1/u_i) = \frac{V_1 + V_2}{LQ}$$

hence,

$$\bar{t}_S = \frac{V_1 + V_2}{Q} \tag{6}$$

Since elution volume is $(\bar{t}_S Q)$ the elution volume for a very small molecule, V_S, is

$$V_S = V_1 + V_2 \tag{7}$$

This is what is found from experiment and what would be expected from flow through any packed bed.

For a molecule approximately the same size as a pore, the probability of entering the pore will be less than that of a small molecule. If the ratio of the probabilities of a large molecule entering to that of a small one is P_e, then the probability of a large molecule flowing through the small pore is

$$\frac{u_1 \frac{V_1}{L}}{Q} P_e \tag{8}$$

And the probability of flowing through a large pore is

$$\frac{u_2 \frac{V_2}{L}}{Q} + \frac{u_1 \frac{V_1}{L}}{Q}(1 - P_e) \tag{9}$$

The resulting elution volume for any molecule, V_m, is then given by

$$V_m = V_1 P_e + V_2 + \frac{u_1}{u_2} V_1 (1 - P_e) \tag{10}$$

When $P_e = 1$, Eq. (10) reduces to Eq. (7) and when $P_e = 0$, as for molecules larger than the small pores, it becomes

$$V_L = V_2 + \frac{u_1}{u_2} V_1 \tag{11}$$

For most cases, $u_2 \gg u_1$ since $r_2 \gg r_1$; hence

$$V_L = V_2 \tag{12}$$

Experimentally this is what has been found; the elution volume for large molecules is equal to the interstitial void volume. Most experimenters calculate the relative exclusion volume as defined by

$$K_e = \frac{V_m - V_L}{V_s - V_L} \tag{13}$$

Upon substituting Eqs. (7) and (12) into Eq. (13), it is found that Eq. (14) is approximately true and the relative exclusion volume is just P_e.

$$K_e = P_e = \frac{V_m - V_2}{V_1} \tag{14}$$

From the definition of P_e it is evident that this quantity is just a sieve constant similar to that used in ultrafiltration. Ferry [61] develops the following expression for the ratio of the probability of a molecule of radius r_0 entering a pore of radius r_1 to that of a molecule much smaller than the pore size r_1.

$$P_e = 2\left(1 - \frac{r_0}{r_1}\right)^2 - \left(1 - \frac{r_0}{r_1}\right)^4 \tag{15}$$

An assumption for this equation is that Brownian motion is negligible. This would be true for the larger pores. However, for the smaller pores, calculations indicate Brownian motion would be the primary determining factor. In this case the equation to use is

$$P_e = \left(1 - \frac{r_0}{r_1}\right)^2 \tag{16}$$

as derived for restricted diffusion, e.g., Pappenheimer [62]. Thus a good estimate of P_e is

$$P_e = (1 + \alpha)\left(1 - \frac{r_0}{r_1}\right)^2 - \alpha\left(1 - \frac{r_0}{r_1}\right)^4 \tag{17}$$

This formula assumes that the two effects are additive; for small pore radius, $\alpha = 0$, and for large, $\alpha = 1$. This assumption should not cause serious error since plots of Eq. (17) for $\alpha = 0$ and $\alpha = 1$, shown in Fig. 2, indicate that P_e does not depend strongly on α.

Pappenheimer [62] multiplies by an additional factor, derived from Stokes flow [63] through a tube. This factor predicts a particle velocity of zero through the pore for molecules of nearly the same size as the pore. Actually a molecule of this size would travel at the velocity of the fluid in such a pore and hence the above factor is not applicable [63]. Also, because of the variation in pore size with length, the radius which determines entrance into the pore is probably different than that which determines fluid velocity with the latter being larger.

Using the equality of P_e and K_e (Eq. 14), one finds the relative exclusion volume to be determined by the formula

$$K_e = (1 + \alpha)\left(1 - \frac{r_0}{r_1}\right)^2 - \alpha\left(1 - \frac{r_0}{r_1}\right)^4 \tag{18}$$

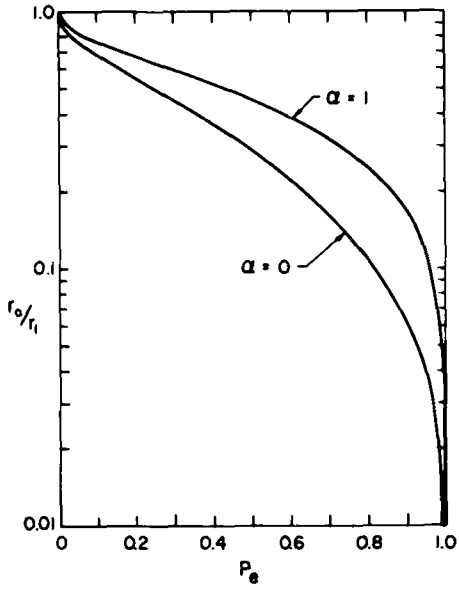

Fig. 2. Relative exclusion volume, P_e, vs molecular to pore radius ratio, r_0/r_1, from Eq. (17) for $\alpha = 1$ and $\alpha = 0$.

where r_0 is the hydrodynamic radius of a particular solute molecule, r_1, is the "average" pore radius in the glass beads, and α is a parameter which is dependent upon r_1 and varies between 0 and 1.

EFFECT OF FLOW RATE THROUGH THE BED ON ELUTION VOLUME

The separation phenomena proposed herein is caused by the factor P_e; thus the effect of flow rate on the separation is really the effect of flow rate on this factor. For small pores where Brownian motion determines the entrance to the pores, no effect of flow rate would be expected, i.e., P_e is independent of flow rate. This is because Brownian motion is unaffected by the low shear flow fields found in GPC separation.

However, for the larger pores when the entrance probability ratio, P_e, is dependent upon the flow in the entrance area, one would expect P_e to be affected by volumetric flow rate through the bed. Since the fractionation mechanism proposed for larger pores is hydrodynamic, some of the well-known dimensional analysis methods of hydrodynamics can be applied to qualitatively explain the result of flow rate variation.

If the flow patterns (fluid streamlines) through the bed are exactly the same for two different flow rates, the elution volumes for each species should remain the same. In other words each molecule of each species for both flow rates will have passed through exactly the same sequence of pores and hence will elute through the bed in the same volume. The flow patterns in the bed are determined by the Navier-Stokes equation given below in dimensionless form [64].

$$\frac{D\bar{v}}{Dt} = \nabla p + \left(\frac{1}{Re}\right)\nabla^2\bar{v} + \left(\frac{1}{Fr}\right)\frac{\bar{g}}{g} \tag{19}$$

If the velocity is small, both the Reynolds (Re) and Froude numbers (Fr) are small and the terms on the right-hand side of the equation dominate. But the resulting equation is linear and the streamlines derived from it for the various flow rates will be exactly the same [63]. Hence any separation performed at low enough flow rates will give the same elution volumes. Only as Re increases will the elution volume for particular polymers start to deviate because the nonlinear inertial terms, $D\bar{v}/Dt$, become important. In this case one would predict a deviation toward lower elution volumes because the inertia of the fluid will tend to carry molecules past the pore openings, giving rise to lower elution volumes.

COMPARISON WITH GPC EXPERIMENTS

Most of the data on GPC have been obtained from columns packed with partially cross-linked polystyrene or dextran (Sephadex) gels of wide pore size distribution and empirically explained; hence, they are not very useful for comparison with the simple theory presented. Data taken by certain investigators [33, 35, 36] allow quantitative comparison with the above predictions and the rest give qualitative supporting evidence for the mechanism and theory.

The relative exclusion volume given by Eq. (16) is related to the polymer solute molecular weight, M, through

$$r_0 = \left(\frac{K_\theta M^{3/2}}{\Phi_\theta}\right)^{1/3} \tag{20}$$

valid for theta conditions [65]. Here Φ_θ is the universal viscosity constant for flexible linear macromolecules and K_θ is the coefficient in the Mark-

Houwink-Sakurada (MHS) intrinsic viscosity equation [65]. Substituting Eq. (20) into Eq. (16) for the hydrodynamic radius yields

$$K_e = \left[1 - \left(\frac{K\theta}{\Phi\theta} \right)^{1/3} \left(\frac{1}{r_1} \right) (M)^{1/2} \right]^2 \qquad (21)$$

Thus the simple theory predicts that the relative exclusion volume, at theta conditions, depends only on the solute molecular weight and the pore radius.

Figure 3 shows ln M vs K_e for the values of r_1 corresponding to those available under the trade name Bio-Glas (Bio-Rad Labs.). The values of $K\theta$ and $\Phi\theta$ used in the calculation of K_e were 8.0×10^{-4} and 2.1×10^{21} (in cgs units), respectively [65]; the values of r_1 are shown next to the curves.

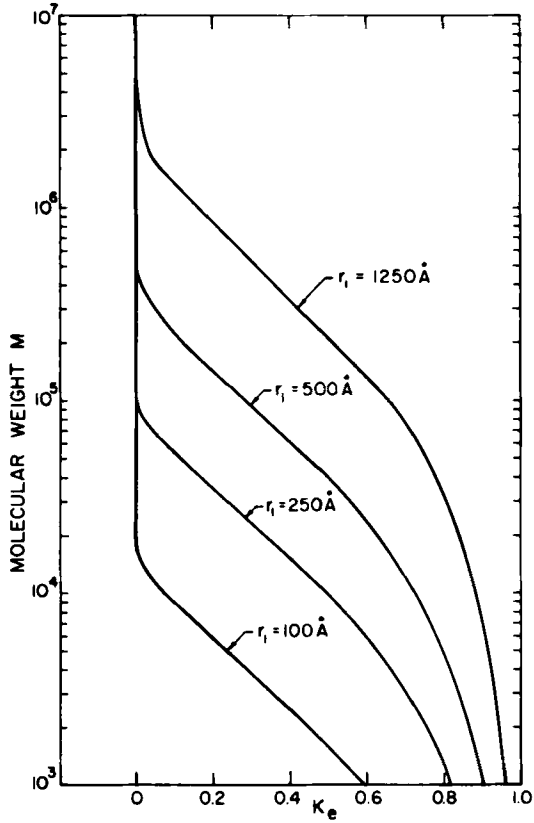

Fig. 3. Molecular weight vs relative exclusion volume from Eq. (21).

The shape of the curves is the same as that observed by Cantow and Johnson [35] and Moore and Arrington [33], but somewhat sharper than those obtained by Yau [36]. Adequate testing of the simple theory would require GPC data for monodisperse polymer fractions in a bed of a uniform pore size operating at theta conditions and in the absence of both internal and external dispersive effects [48-51, 57, 58].

Since experimental data meeting all these conditions are not at present available, we have chosen the data of Moore and Arrington [33], Cantow and Johnson [35], and Yau [36] for comparison with the theory, because they come the closest to meeting the first two requirements.

The use of the equivalent hydrodynamic radius, r_0, to characterize macromolecular size has recently been reviewed by Boni et al. [24]. They conclude, on the basis of their results and those of Grubisic et al. [12, 14], Benoit et al. [11], and Wild and Guliana [13], that r_0 is the preferred calibration parameter for most polymers. The equation,

$$r_0 = \left(\frac{KM^{a+1}}{\Phi} \right)^{1/3} \tag{22}$$

based on the comprehensive reviews of Tanford [66], Tompa [67], Morawetz [68], and Birshtein and Ptitsyn [69], where K and a are the coefficients in the MHS intrinsic viscosity equation and Φ depends on solvent power and molecular weight for nontheta conditions [65-69], was used to calculate r_0 in Eq. (16).

Due to the lack of an exact expression for the dependence of Φ on molecular weight and solvent power, the values of K, a, and Φ were estimated from the extensive listing of Kurata and Stockmayer [70]. However, the actual values chosen for K, a, and Φ are not critical to the analysis of the simple theory presented here because small variations in these numbers do not have a significant effect upon the predicted GPC results.

Figure 4 shows the elution volumes of various polystyrenes vs their molecular weights on two porous glasses of different pore sizes with an organic solvent as eluant. The experimental data were obtained by Moore and Arrington [33] on glasses supplied by Haller [32]. The solid lines shown were calculated from our theory. The values of $\Phi = 2.1 \times 10^{21}$ cgs, $K = 8.0 \times 10^{-4}$ cgs, and $a = 0.5$ were used in the calculation of r_0. V_2 and V_1 were estimated from the experimental data as 26.5 and 20.0 cm^3, respectively. The values of r_1 used in Eq. (16) were 400 and 3600 Å as opposed to the mercury intrusion values of 121 and 900 Å. However, as pointed out by Haller [32], electron micrographs of the beads revealed that the effective pore radius is roughly three times the mercury intrusion

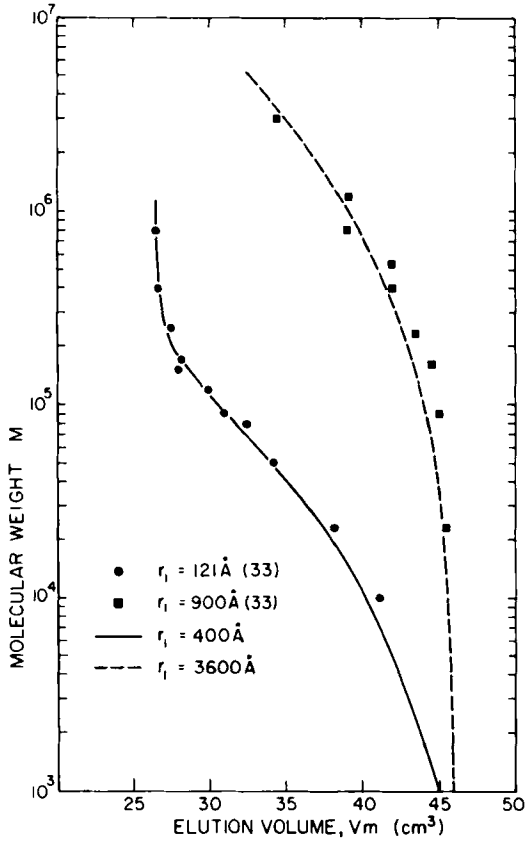

Fig. 4. Comparison of the theoretical prediction with the experimental GPC data of Moore and Arrington [33] on porous glass beads.

value. Thus, the chosen values are consistent with the available knowledge.

Figure 5 shows the elution volumes of various polystyrene fractions vs their molecular weights on two porous glasses (Bio-Glas 500 and 1000) with toluene as the eluant. The experimental data were obtained by Cantow and Johnson [35] and the lines calculated using Eqs. (16) and (22). The values of $\Phi = 2.87 \times 10^{21}$, $K = 13.4 \times 10^{-5}$, and $a = 0.71$ were used in Eq. (22). V_2 and V_1 were estimated, from the experimental data, to be 27.5 and 9.0 cm^3, respectively. The values of r_1 used in Eq. (16) were 500 and 1000 Å, just double the commercially recommended values.

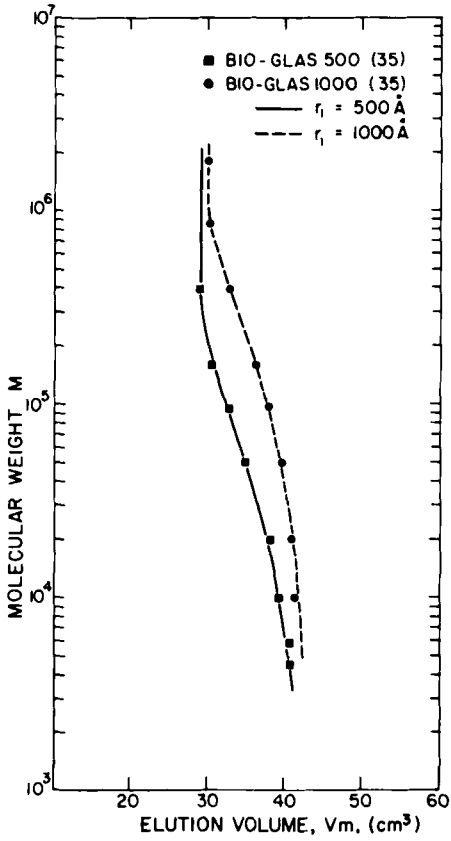

Fig. 5. Comparison of the theoretical prediction with the experimental GPC data of Cantow and Johnson [35] on porous glass beads.

Figure 6 shows the relative exclusion volume of various polystyrenes vs their molecular weights on Bio-Glas 200 with chloroform as the eluant. The experimental data were obtained by Yau [36] and the solid line calculated from the theory. The values of $\Phi = 2.87 \times 10^{21}$, $K = 11.2 \times 10^{-5}$ and $a = 0.73$ were used in the calculation of r_0. The value of r_1 used was 200 Å, just double the average mercury intrusion value. The theory probably predicts a sharper separation curve than observed by Yau [36] because the relationship between molecular weight and hydrodynamic radius is not reliable for $M < 10^4$ [65-70]. Cantow and Johnson [34] have shown the

Bio-Glas beads to have a rather broad pore-size distribution which may further complicate predictions of elution volume for small polymer sizes.

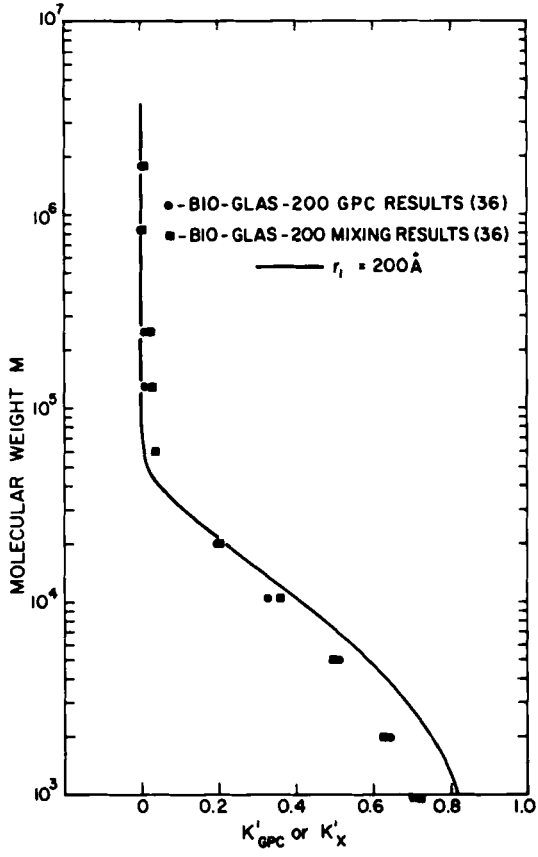

Fig. 6. Comparison of the theoretical prediction with the experimental GPC data of Yau [36] on porous glass beads.

As can be seen, this theory successfully predicts the shape of the elution volume curve and quantitatively agrees with the data of three investigators who used glass beads for GPC packing in their experiments. In these predictions only one parameter, r_1, the radius of the pores in the packing, was used, and its choice is in the range one would expect from other measurements [32, 34]. Thus it appears that the theory can be used quantitatively in these cases.

Presently, most of the applications of GPC involve the use of cross-linked polymers as the packing because they are easy to use, available commercially, and give a larger molecular weight range of separation. However, as stated previously, this theory would not be expected to give quantitative agreement because of the broad distribution of pore sizes expected in these packings. To give some indication of the discrepancies, the GPC data of Yau [36] for various polystyrenes in chloroform on polystyrene gel (Waters 10^4 Å designation) at two different flow rates were plotted in Fig. 7 and compared with Eq. (18) with $\alpha = 1$ and $\alpha = 0$. The solid line shown was calculated from Eq. (18) using $V_2 = 27$ ml, $V_1 = 23$ ml, $r_1 = 500$ Å, and

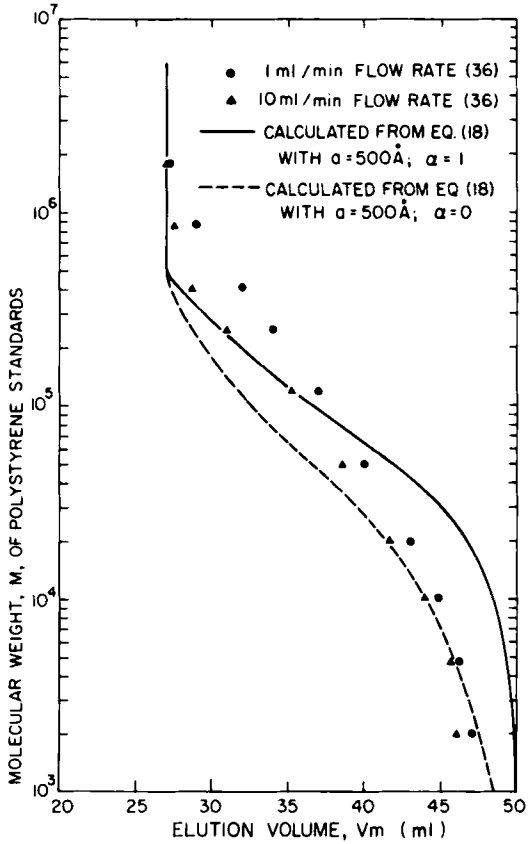

Fig. 7. Comparison of the theoretical prediction with the experimental GPC data of Yau [36] on cross-linked polystyrene gel (10^4 Å designation).

$\alpha = 1$. The dashed line was calculated from Eq. (18) using $\alpha = 0$ and the same values for V_2, V_1, and r_1. The parameter values used to calculate r_0 were the same as those used to calculate the curve in Fig. 6. As expected, the fit is fairly poor compared to the experimental data on porous glass (Fig. 6), indicating the need for a more general theory. From the value of the pore radius which is required to fit the data ($r_1 = 500$ Å), one would expect a value of α near 0, but because of the large range of pore sizes, it probably varies between 0 and 1. Also the designation of 10^4 Å does not seem to be the pore size range that is actually responsible for the separation.

In the previous discussions the values of α chosen were always 0 or 1; a choice of an intermediate value might give a better fit; specifically in the case of Moore and Arrington [33]. Since experimental inaccuracies exist and approximation formula were used to determine the hydrodynamic radius of the molecule, no better choice of α appeared warranted.

COMPARISON WITH STATIC EXPERIMENTS

Several experimenters [36, 42] have investigated the GPC process by performing static experiments to determine the volume exclusion and correlating this with the separation found in the GPC column. Both Yau [36] and Ackers [42] found reasonable agreement between the static experiments and the GPC separation for packing containing small pores, but deviations appeared as the packing pore size became larger. Both experimenters then used diffusion theories to explain their deviations.

The theory presented herein will be used to interpret these results, specifically for the data of Yau [36]. Yau used the following equation (his terminology) to relate the equilibrium distribution coefficient, K_X, to the ratio of the initial concentration, C_i, to final concentration, C_O,

$$\left[1 - (C_i/C_O)\right] = \frac{V_g}{V_i}\left[1 - K_X\right] \tag{23}$$

where V_i is the initial liquid volume and V_g the liquid volume inside the porous substrate. This distribution coefficient is defined as the ratio of the average concentration of solute inside the pores, C_g, to those outside the pores C_O. Haller [32] suggests that this ratio K_X should have the following form for molecules having radii of the same order of magnitude as the radius of the pore, r_1.

$$K_X = \left(1 - \frac{r_0}{r_1}\right)^2 \tag{24}$$

But from our previous discussion we found that

$$K_{GPC} = K_e = (1 + \alpha)\left(1 - \frac{r_0}{r_1}\right)^2 - \alpha\left(1 - \frac{r_0}{r_1}\right)^4 \tag{25}$$

For small pores it is known that α should equal zero. For this case, K_{GPC}, should give a linear plot vs the quantity $[1 - (C_i/C_0)]$; this linearity was found for the small pores in the glass beads used by Yau (see his Fig. 3 [36]). For larger pores, however, α no longer is zero; for the case of $\alpha = 1$ the following relationship for K_e and $[1 - (C_i/C_0)]$ is obtained by combining Eqs. (23-25)

$$K_e = 1 - \left(\frac{V_i}{V_g}\right)^2 \left[1 - \frac{C_i}{C_0}\right]^2 \tag{26}$$

The resulting curve is plotted in Fig. 8 with the parameter (V_i/V_g) chosen to be 3.77 from Yau's Fig. 4. The GPC data of Yau are also plotted on this graph and the agreement is good. A choice of α slightly less than 1 probably would give a better fit.

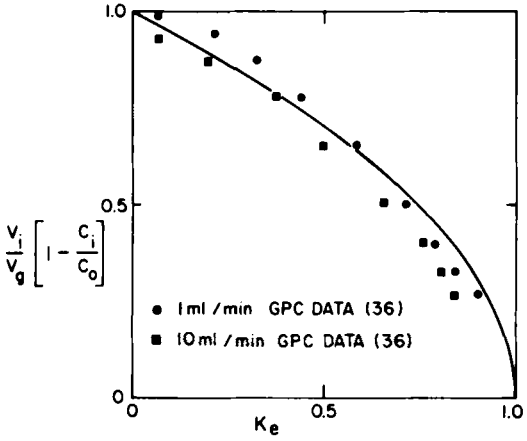

Fig. 8. Theoretical comparison of equilibrium mixing experiment. GPC data of Yau [36] on cross-linked polystyrene gel of 10^4 Å designation.

The close fit in this last case may be somewhat fortuitous because the derivation involved the assumption that the restriction radius, r_1, for flow through the separation column is the same as the radius used to determine the equilibrium concentration. In practice the radius for equilibrium is probably larger than r_1.

Work on the dispersion effects and methods of dispersion correction is continuing using this simple theory. A general theory for columns containing a distribution of pore sizes in the packing particles as well as a random packing of the particles in the bed is being developed.

NOMENCLATURE

a	coefficient in the MHS equation
Å	Ångstrom (10^{-8} cm)
C_g	concentration inside the pores (g/cm^3)
C_i	initial concentration (g/cm^3)
C_0	final concentration (g/cm^3)
Fr	Froude number
$\bar{g}$	gravitational acceleration vector (cm/sec^2)
K_{GPC}	GPC distribution coefficient
K_e	relative exclusion volume; defined by Eq. (13)
K_θ	coefficient in the MHS equation
K_x	equilibrium distribution coefficient
L	length of the bed (cm)
M	solute molecular weight (g/g-mole)
N	number of holes
∇P	dimensionless pressure gradient
P_1	probability of molecule entering a small pore; defined by Eq. (1)
P_2	probability of molecule entering a large pore; defined by Eq. (2)
P_e	ratio of probability of large molecule entering a pore to that of a solvent molecule entering the pore
Q	total volumetric flow rate (cm^3/sec)
r_1	pore radius in glass beads (cm)
r_2	interstitial pore radius between glass beads (cm)
r_0	hydrodynamic radius of solute molecule (cm)
Re	Reynolds number
t	dimensionless time

t_S	total time spent in the bed (sec); defined by Eq. (4)
$\bar{t_S}$	average value of t_S; defined by Eq. (5)
$\bar{v}$	dimensionless velocity vector
u_1	velocity through the small pores (cm/sec)
u_2	velocity through the large pores (cm/sec)
V_1	volume of small pores in the bed (cm^3)
V_2	volume of large pores in the bed (cm^3)
V_S	elution volume of small molecule (cm^3)
V_m	elution volume of any molecule (cm^3)
V_L	elution volume of large molecule (cm^3)
V_g	liquid volume inside the porous substrate (cm^3)
V_i	initial liquid volume (cm^3)
α	dimensionless parameter defined by Eq. (17)
Φ_θ	universal viscosity constant

REFERENCES

[1] K. H. Altgelt and J. C. Moore, *Polymer Fractionation*, (M. J. R. Cantow, ed.), Academic, New York, 1967.

[2] J. F. Johnson, R. S. Porter, and M. J. R. Cantow, *Rev. Macromol. Chem.*, **1**, 393 (1966).

[3] H. Determann, *Gel Chromatographie*, Springer, Heidelberg, 1967.

[4] H. Determann, *Angew. Chem. Intern. Ed.*, **3**(9), 608 (1964).

[5] J. Cazes, *J. Chem. Educ.*, **43**, 567 (1966).

[6] K. H. Altgelt, in *Advan. Chromatogr.*, Vol. 7 (J. C. Giddings and R. A. Keller, eds.), Dekker, New York, 1968.

[7] B. Gelotte, in *New Biochemical Separations* (A. T. James and L. J. Morris, eds.), Van Nostrand, Princeton, New Jersey, 1963.

[8] M. J. R. Cantow, R. S. Porter, and J. F. Johnson, *J. Polym. Sci., Part A-1*, **5**, 987 (1967).

[9] K. A. Boni, F. A. Sliemers, and P. B. Stickney, *J. Polym. Sci., Part A-2*, **6**, 1579 (1968).

[10] M. Lepage, R. Beau, and A. J. Devries, *J. Polym. Sci., Part C*, **21**, 119 (1968).

[11] H. Benoit et al., *J. Chim. Phys.*, **63**, 1507 (1966).

[12] Z. Grubisic, L. Reibel, and G. Spach, *C. R. Acad. Sci., Paris, Ser. C.*, **246**, 1690 (1967).

[13] L. Wild and R. Guliana, *J. Polym. Sci., Part A*, **2**, 1087 (1967).

[14] F. Grubisic, P. Rempp, and H. Benoit, *J. Polym. Sci., Part B*, **5**, 753 (1967).

[15] L. H. Tung, *J. Appl. Polym. Sci.*, **10**, 375, 1271 (1966).
[16] J. H. Duerksen and A. E. Hamielec, *Polym. Preprints*, **8**, 1338 (1967).
[17] L. H. Tung, J. C. Moore, and G. W. Knight, *J. Appl. Polym. Sci.*, **10**, 1261 (1966).
[18] M. Hess and R. F. Kratz, *J. Polym. Sci., Part A*, **2**, 731 (1966).
[19] W. N. Smith, *J. Appl. Polym. Sci.*, **11**, 639 (1967).
[20] H. E. Pickett, M. J. R. Cantow, and J. F. Johnson, *J. Polym. Sci., Part C*, **21**, 67 (1968).
[21] J. L. Waters, *Polym. Preprints*, **6**, 1061 (1965).
[22] H. E. Adams, K. Farhat, and B. L. Johnson, *Ind. Eng. Chem., Prod. Res. Develop.*, **5**, 126 (1966).
[23] M. J. R. Cantow, R. S. Porter, and J. F. Johnson, *J. Polym. Sci., Part B*, **4**, 707 (1966).
[24] K. A. Boni, F. A. Sliemers, and P. B. Stickney, *J. Polym. Sci., Part A2*, **6**, 1567, 1579 (1968).
[25] J. C. Moore, *J. Polym. Sci., Part A*, **2**, 835 (1964).
[26] G. Meyerhoff, *Ber. Bunsenges, Phys. Chem.*, **69**, 866 (1965).
[27] W. B. Smith and A. Kollmansberger, *J. Phys. Chem.*, **69**, 4157 (1965).
[28] F. Rodriguez, R. A. Kulakowski, and O. K. Clark, *Ind. Eng. Chem., Prod. Res. Develop.*, **5**, 121 (1966).
[29] M. F. Vaughan, *Nature*, **195**, 801 (1962).
[30] W. Haller, *J. Chem. Phys.*, **42**, 686 (1965).
[31] W. Haller, *Nature*, **206**, 693 (1965).
[32] W. Haller, *J. Chromatogr.*, **32**, 676 (1968).
[33] J. C. Moore and M. C. Arrington, Paper presented at Intern. Symp. Macromol. Chem., Tokyo, 1966.
[34] M. J. R. Cantow and J. F. Johnson, *J. Polymer, Sci., Part A-1*, **5**, 2835 (1967).
[35] M. J. R. Cantow and J. F. Johnson, *J. Appl. Polym. Sci.*, **11**, 185 (1967).
[36] W. W. Yau, *J. Polym. Sci., Part A-1*, **7**, 483 (1969).
[37] P. Flodin, Dissertation, Univ. Uppsala, 1962.
[38] J. Porath and P. Flodin, *Nature*, **183**, 1657 (1959).
[40] P. G. Squire, *Arch. Biochem. Biophys.*, **107**, 471 (1964).
[41] T. C. Laurent and J. Killander, *J. Chromatogr.*, **14**, 317 (1964).
[42] G. K. Ackers, *Biochemistry*, **3**, 723 (1964).
[43] R. E. Treybal, *Mass-Transfer Operations*, 2nd ed., McGraw-Hill, New York, 1968.
[44] J. Hutchings and J. J. Carberry, *Amer. Inst. Chem. Eng. J.*, **12**, 30 (1966).

[45] J. J. Carberry and D. White, *Ind. Eng. Chem.*, **61**(7), 27 (1969).
[46] W. W. Yau and C. P. Malone, *J. Polym. Sci., Part B*, **5**, 663 (1967).
[47] E. F. Casassa, *J. Polym. Sci., Part B*, **5**, 773 (1967).
[48] J. B. Carmichael, *J. Polym. Sci., Part A-2*, **6**, 517 (1968).
[49] J. B. Carmichael, *Polym. Preprints*, **9**, 572 (1968).
[50] J. B. Carmichael, *J. Chem. Phys.*, **49**, 5161 (1968).
[51] J. B. Carmichael, *Macromolecules*, **1**, 526 (1968).
[52] K. O. Pederson, *Arch. Biochem. Biophys. Suppl.*, **1**, 157 (1962).
[53] R. L. Steere and G. K. Ackers, *Nature*, **196**, 475 (1962).
[54] E. Heftmann, ed., *Chromatography*, Reinhold, New York 1961, p. 406.
[55] D. M. W. Anderson and J. F. Stoddart, *Anal. Chim. Acta*, **34**, 401 (1966).
[56] R. L. Pecsok and D. Saunders, in *Separation Techniques in Chemistry and Biochemistry* (R. A. Keller, ed.), Dekker, New York, 1967.
[57] F. W. Billmeyer, G. W. Johnson, and R. N. Kelley, *J. Chromatogr.*, **34**, 316, 322 (1968).
[58] R. N. Kelley and F. W. Billmeyer, *Anal. Chem.*, **41**, 874 (1969).
[59] K. B. Bischoff, *Ind. Eng. Chem.*, **58**, 18 (1966).
[60] K. B. Bischoff, *Chem. Engr. Sci.*, **24**, 607 (1969).
[61] J. D. Ferry, *J. Gen. Physiol.*, **20**, 95 (1936).
[62] J. R. Pappenheimer, *Physiol. Revs.*, **33**, 387 (1953).
[63] J. Happel and H. Brenner, *Low Reynolds Number Hydrodynamics*, Prentice-Hall, Englewood Cliffs, New Jersey, 1965.
[64] R. B. Bird, W. E. Stewart, and E. N. Lightfoot, *Transport Phenomana*, Wiley, New York, 1960.
[65] P. J. Flory, *Principles of Polymer Chemistry*, Cornell Univ. Press, Ithaca, New York, 1953.
[66] C. Tanford, *Physical Chemistry of Macromolecules*, Wiley, New York, 1965.
[67] H. Tompa, *Polymer Solutions*, Butterworths, London, 1956.
[68] H. Morawetz, *Macromolecules in Solution*, Wiley (Interscience), New York, 1965.
[69] T. M. Birshtein and O. B. Ptitsyn, *Confromations of Macromolecules*, Wiley (Interscience), New York, 1966.
[70] M. Kurata and W. H. Stockmayer, *Fortschr. Hochpolymer. Forsch.*, **3**, 196 (1963).

Accepted by editor October 16, 1969
Received for publication December 23, 1969