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SEPARATION BY FLOW AND ITS APPLICATION  
TO GEL PERMEATION CHROMATOGRAPHY

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## SUMMARY

When dilute solutions of finite size particles undergoing Brownian motion flow through a capillary, the larger particles have higher average velocities than the smaller particles. Thus one can obtain a separation of particles of different sizes due to fluid flow. The elution volumes of suspended particles or polymer molecules are derived for various tube geometries. Following TAYLOR, the effects of diffusional broadening of the volume elution peak for finite size particles are discussed and the process is shown to be chromatographic.

Models of a gel permeation chromatographic column are proposed in which there is fluid and particle flow through each of the beads as well as around them. Diffusion is allowed within and outside of the beads. Equations for the location of the volume elution peaks are computed for such models and shown to yield functional dependence on the polymer radius and column geometry very much like equations derived by previous workers for models of gel permeation chromatographic columns in which there was no flow allowed within the beads. Explicit formulae are given for the second and third moments for the above models. It is shown that for a monodisperse species the volume elution peak is always a gaussian of a finite width. It is shown that beads with open pores that allow for flow always have better separation capabilities than beads with pores that do not allow for flow.

## I. INTRODUCTION

An isolated polymer molecule flowing down the inside of a thin capillary and undergoing Brownian motion will have an average velocity greater than that of the solvent. This is because the center of the particle (assumed to be a rigid sphere) cannot get any closer to the walls of the capillary than its radius. It therefore samples only those solvent velocities away from the walls. Since the solvent velocity is larger, the farther the distance from the wall, larger molecules will have larger average velocities than smaller molecules. See Fig. 1.

Suppose we now introduce particles of two different sizes simultaneously at the top of the column. The average distance between these particles will increase linearly with time as they flow through the tube because they have different average velocities. On the other hand the peak widths of the distribution of particle distances about

their mean value for each kind of particle increases as the square root of time. This is a characteristic of particle diffusion. It therefore follows that by waiting for sufficiently long periods of time the separation between peaks can be made large compared to the width of the peaks. The particles therefore separate into two groups.

These ideas have been placed on a firm quantitative foundation in a previous paper<sup>1</sup>. The main results for a single capillary are recapitulated in Section 2.

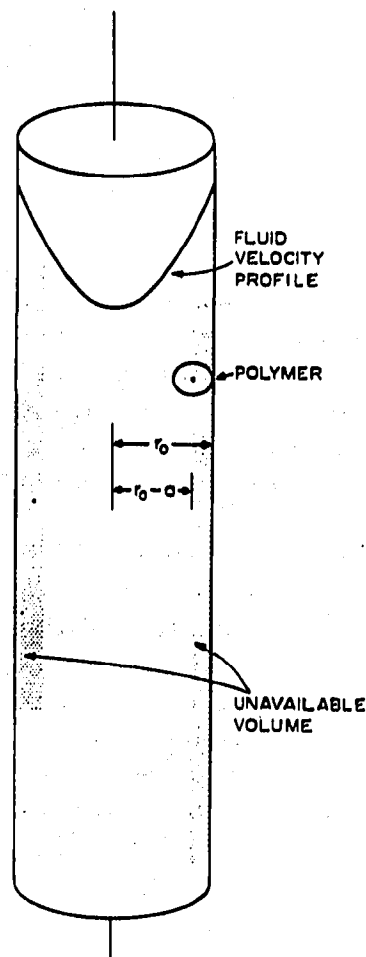


Fig. 1. Schematic of flow down a single tube. The fluid velocity profile causes polymer separation by virtue of the unavailable volume.

Because the elution characteristics of a single capillary are very much like those of gel permeation chromatographic (GPC) columns we were led to consider the elution properties of various networks of capillaries (combinations of capillaries in series and in parallel). The main results are summarized in Section 3. A complete paper on this aspect of the problem has appeared elsewhere<sup>2</sup>.

A particular combination of tubes is considered as a model for GPC (see Fig. 2). In this model there are tubes of two diameters. The large tubes represent flow between the beads (interstitial flow) of a GPC column, and the small tubes represent flow through (intra-bead-flow) the beads. The idea that there is flow within the bead as well as around it is essential to the applicability of the separation by flow (SBF) concept to GPC. In Section 3 we also discuss the application of SBF to GPC.

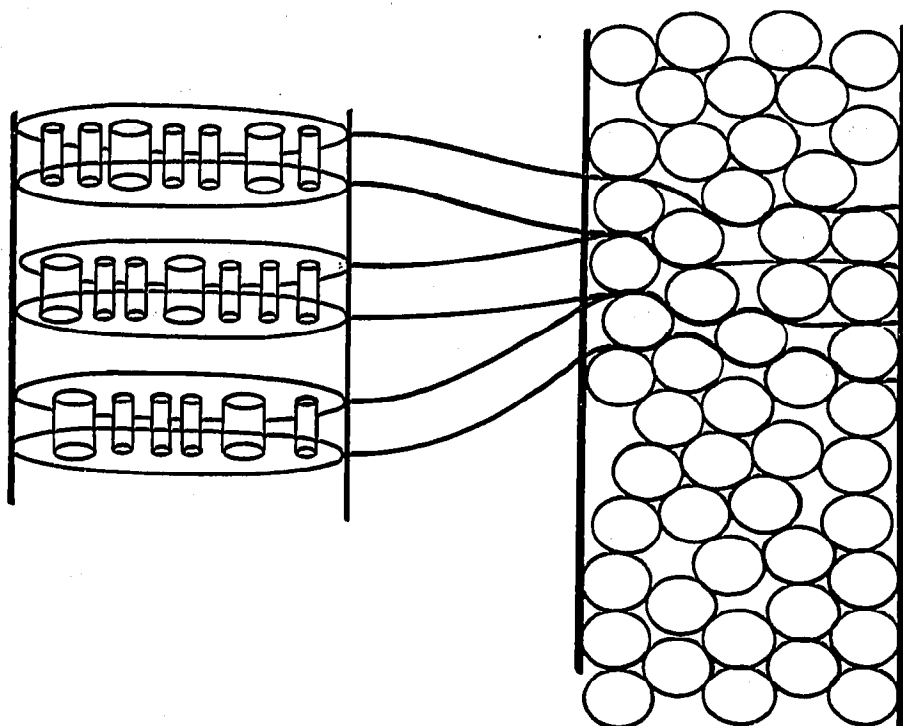


Fig. 2. The bank model of a GPC column. The large tubes in a given bank (on left) represent the totality of interstitial regions at that same level in the column (on right). The small tubes in this bank represent the totality of fine tubes within the beads at this same level. The space between banks serves as a mixing region and is not considered to have any volume.

## 2. SEPARATION BY FLOW IN A LONG THIN CAPILLARY

### A. Volume elution

The velocity  $v_f$  of solvent flowing down a tube of radius  $r_0$  under Poiseuille flow is

$$v_f = \frac{1}{4\eta} \frac{\partial p}{\partial l} (r_0^2 - r^2) \quad (1)$$

The velocity of the center of mass of a polymer molecule,  $v_p$ , has been derived under the free draining assumption<sup>1</sup>.

$$\frac{v_f - v_p}{u_0} = \frac{2}{3} \left( \frac{s}{r_0} \right)^2 \quad (2)$$

where  $u_0$  is the fluid velocity along the axis of the capillary.

The radius of gyration is  $s$ . It was found that the molecule does not migrate radially (unless there is diffusion), but is retarded by the amount in eqn. 2. HAPPEL AND BYRNE<sup>3</sup> have derived a relation for the slip at the axis of a cylindrical tube of radius  $r_0$  for rigid impermeable spheres of radius  $s$ . They obtain

$$\left[ \frac{v_f - v_p}{v_f} \right]_{r=0} = \frac{2}{3} \left( \frac{s}{r_0} \right)^2 \quad (3)$$

This relation is identical with ours if we identify the radius of the sphere with the radius of gyration of the molecule. Eqn. 3 has been confirmed experimentally by GOLDSMITH AND MASON<sup>4</sup>. We can now evaluate the average velocity and therefore the elution volume. Because of entropy forces a polymer molecule will tend to maintain its shape as it flows down the tube. That is to say, if we deform a polymer molecule from its spherical shape by means of an external force, a restoring force is set up by the polymer which acts in a direction so as to restore the shape. It is for this reason that the center of mass of the polymer molecule cannot approach too closely the walls of the tube down which it flows. We are thus led to define an effective radius,  $a$ , for the polymer. For flexible polymer molecules it has been shown that this radius is related to the radius of gyration,  $s$ , by

$$a = \frac{3}{\sqrt{\pi}} s \quad (4)$$

If Brownian motion in a direction perpendicular to the axis of the tube is allowed, then the center of mass of the polymer molecule will sample every possible horizontal position in the tube with equal probability, except for the fact that it cannot be closer than a distance,  $a$ , to the wall. We obtain then for  $\langle v_p \rangle$ , the average velocity of the polymer, in a circular cylinder

$$\begin{aligned} \langle v_p \rangle &= u_0 \int_0^{r_0-a} \left[ 1 - \left( \frac{r_c}{r_0} \right)^2 - \gamma \left( \frac{a}{r_0} \right)^2 \right] r_c dr_c / \int_0^{r_0-a} r_c dr_c \\ \langle v_p \rangle &= u_0 \left[ 1 - \frac{(1 - a/r_0^2)}{2} - \gamma \left( \frac{a}{r_0} \right)^2 \right] \quad \text{circular cylinders} \end{aligned} \quad (5)$$

where

$$u_0 = \frac{\partial p}{\partial l} \frac{r_0^2}{4\eta}$$

We have let  $r_0$  be the radius of the cylinder and  $r_c$  the distance from an axis down the center of the tube to the particle center of mass. It should be noted that  $u_0$  as defined in eqn. 5 is the maximum velocity of the fluid in the tube. The  $\gamma$  in eqn. 5 is defined by

$$\gamma a^2 = 2s^2/3 \quad (6)$$

For flexible polymer molecules  $\gamma = 2\pi/27$ . However, in general,  $\gamma$  is a function of the shape of the particle as well as the density distribution within the particle. For this reason we retain  $\gamma$  as a parameter. In a similar manner we obtain for parallel plates

$$\langle v_p \rangle = u_0 \left[ 1 - \frac{(1 - a/z_0^2)}{3} - \frac{\gamma}{2} \left( \frac{a}{z_0} \right)^2 \right] \quad \text{plates} \quad (7)$$

where for parallel plates

$$u_0 = \frac{\partial p}{\partial l} \frac{z_0^2}{2\eta}$$

The elution volume,  $\bar{V}_e$ , is the fluid volume flow rate,  $Q$ , times the time needed for passage of the polymer through the capillary,  $t$ . Thus, for a tube of length  $l$

$$\bar{V}_e = Qt = Ql/\langle v_p \rangle \quad (8)$$

We obtain then

$$\bar{V}_e = \frac{\pi l r_0^2}{2 \left[ 1 - \frac{(1-R)^2}{2} - \gamma R^2 \right]} \quad \text{circular cylinders} \quad (9)$$

$$\bar{V}_e = \frac{z_0 l}{\frac{3}{4} \left[ 1 - \frac{(1-R)^2}{3} - \frac{\gamma}{2} R^2 \right]} \quad \text{plates} \quad (10)$$

In eqn. 9,  $R = a/r_0$ , while in eqn. 10 it is  $R = a/z_0$ . Thus, the elution volume is equal to the elution volume of solvent times a factor which is a function only of  $R$  and the geometry of the capillary.

In Fig. 3 the effect of varying  $\gamma$  on the elution volume of a circular cylinder is given; for convenience we have let  $\pi l r_0^2$  equal 1 in this figure. We have plotted  $V_e$  vs.  $3 \log R$  in the figure since  $R^3$  is nearly proportional to the so-called hydrodynamic volume of the polymer. We pointed out previously<sup>5</sup> that the features of the curves given here are similar to the log hydrodynamic volume vs. volume elution curves of GRUBISIC *et al.*<sup>6</sup>, found experimentally for a GPC column. We wish to emphasize here the similarity of the curves for  $R < 0.2$ . Clearly for this region we have an insensitivity to  $\gamma$  in the  $V_e$  vs.  $3 \log R$  plots.

In Fig. 4 we have plotted  $V_e$  vs.  $3 \log R$  for three different cross sections of tubes;

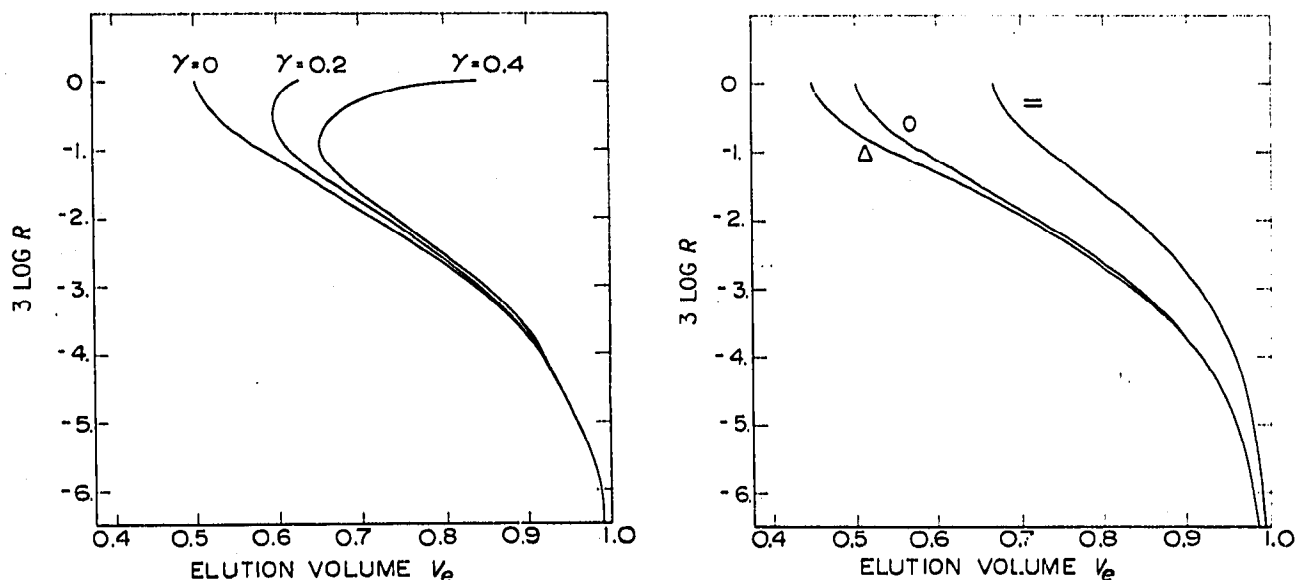


Fig. 3. A plot of the normalized elution volume versus the logarithm of the effective particle volume for separation in a single circular cylinder is shown. The effect of varying  $\gamma$ , the parameter relating to the magnitude of the retardation effect, is seen to be small below  $R = 0.4$ .  $R$  is defined as  $a/r_0$ .

Fig. 4. A plot of the normalized elution volume versus the logarithm of the effective particle volume (with  $\gamma = 0$ ) for various tube geometries for a single tube is shown. Notice the general shape of the curve is insensitive to the geometry.  $R$  is defined as  $a/r_0$ .

we have normalized all the curves to  $V_e = 1$  for  $R = 0$ . In this plot we see that although the parallel plates result is different from the others, the shape of the curves show a general insensitivity to cross section for smaller  $R$ 's

Neglecting the retardation effect ( $\gamma = 0$ ), the ratio of maximum to minimum elution volume is 2 for circular cylinders and 1.5 for parallel plates. It is interesting to compute this ratio for tubes of various other cross sections. This is done by computing the ratio of maximum velocity to average velocity. We obtain, for a square cross section 2.5, for an elliptical cross section 2.0 (independent of the eccentricity of the ellipse), and for equilateral triangle 2.2. Thus, one might expect the effect of different cross section on volume elution is a small one.

### B. Dispersion in the volume elution peak due to diffusion

TAYLOR had addressed himself to the problem of dispersion of soluble matter in solvent flowing through a circular cylinder<sup>7</sup>. ARIS has extended the results to cylinders of arbitrary cross section<sup>8</sup>. Their problem is identical with ours except for one factor: the size of the molecule. They assumed that the soluble matter is made up of point particles, while we assume our soluble matter is made up of particles of finite size.

We have adopted their treatment to the solution of the problem for finite size molecules. The starting point is the three dimensional diffusion equation in a moving medium.

$$D\nabla^2 C = \frac{\partial C}{\partial t} + \vec{v} \cdot \vec{\nabla} C \quad (11)$$

where  $C$  is the concentration of solute particles,  $\vec{v}$  is their velocity,  $D$  their diffusion constant, and  $t$  time. Eqn. 11 is a generalization of the ordinary diffusion equation to a moving medium. The main result of TAYLOR and ARIS can be described as follows. Suppose we define  $C_m$  to be the mean concentration over the cross-sectional area whose normal is parallel to the axis of the cylinder. Then  $C_m$  is found to obey a one dimensional diffusion equation.

$$K \frac{\partial^2 C_m}{\partial x^2} = \frac{\partial C_m}{\partial t} \quad (12)$$

with an effective diffusion coefficient  $K$ ,

$$K = D + \theta \frac{u_0^2 r_0^2}{D} \quad (13)$$

The coordinate system of eqn. 12 moves along with the average velocity of the particle and the direction along the tube is the  $x$  direction.  $\theta$  is a number which depends on the cross sectional geometry and has value 1/192 for circular cylinders and 1/120 for parallel plates. For parallel plates one replaces  $r_0$  by  $z_0$ ; in both cases  $u_0$  is the maximum fluid velocity in the tube. The effective diffusion coefficient  $K$  has an ordinary part  $D$  resulting from longitudinal diffusion and a part  $\theta u_0^2 r_0^2 / D$  which results from transverse diffusion and simultaneous smearing due to the fluid velocity profile. The shear gradient causes material at different levels in the tube to travel at different velocities thereby smearing the particles over large longitudinal distances.

Thus this effect increases with increasing  $u_0$ . However, the rate at which particles sample various levels is important; if the particle sampled quickly ( $D$  large) then there would be only a small amount of smearing since the longitudinal velocity of each particle is the average velocity. However, if the particle sampled the various levels slowly then there would be much smearing. This is the reason for the inverse dependence in  $D$  in the second term of eqn. 13. This term is the dominant one for many situations of interest. Thus, the larger the diffusion coefficient, the smaller the spreading in the elution volume peak!

The only effect of particle size is to scale the quantities  $u_0$  and  $r_0$ , ( $z_0$ ) as follows.

$$r_0 \rightarrow (r_0 - a)$$

$$u_0 \rightarrow u_0 \left( \frac{r_0 - a}{r_0} \right)^2 \text{ circular cylinders} \quad (14)$$

$$z_0 \rightarrow (z_0 - a)$$

$$u_0 \rightarrow u_0 \left( \frac{z_0 - a}{z_0} \right)^2 \text{ parallel plates} \quad (15)$$

Eqns. 12 and 13 are now applicable with the substitution afforded by eqns. 14 and 15.

### C. Implications of the equations and further results

Separation by flow occurs in the capillary considered in sections 2 A and B. The average distance traveled by molecule  $i$  down a tube in time  $t$  is  $\langle v_p \rangle t$ ; then the distance between peak centers for two substances 1 and 2 is

$$d_{12} = |\langle v_p \rangle_1 - \langle v_p \rangle_2| t \quad (16)$$

Because of diffusion, the elution volume peaks become spread out in time. But as is always the case for diffusion, the width of the peaks are proportional to  $\sqrt{t}$  rather than  $t$ . Thus, if one waits for a long enough time, either by using a long tube or equivalently by recycling through one tube, one can always separate the two materials.

From eqns. 9 and 10 we observe that the elution volume peak is a function only of the effective radius of the particle and of the geometry of the tube. It is independent of both viscosity of the fluid and the diffusion coefficient of the molecules. It also does not depend on the pressure head and the flow rate through the tube. If we flow different molecules through the same tube then the only pertinent variable is the effective radius. We have already pointed out<sup>1</sup> that the effective radius cubed of a polymer is nearly proportional to its hydrodynamic volume. Presumably then we have a method of characterizing molecules on the basis of hydrodynamic volume alone. For rigid rods also, the only pertinent variable for volume elution is hydrodynamic volume<sup>1</sup>.

The validity of the concept of separation by flow is independent of the detailed assumptions used in deriving the specific formulas. It is, for example, not necessary for us to have a quadratic dependence of fluid velocity in a circular cylinder. All that is required is that we have on the average a larger velocity toward the center than at the boundary of the tube. It is not necessary even to have Brownian motion. All that is needed is a mechanism whereby the particles sample various levels in the tube.

Turbulence for example might replace Brownian motion as the mode of sampling.

If one attempts to use a bundle of identical capillaries as a practical method of separating polymer molecules of molecular weight  $10^6$  or less one finds that it is impossible to choose reasonable values of the parameters, tube size, numbers, and length. One cannot have both good separation which requires narrow capillaries and large flow which requires wide capillaries. For this reason we were led to the study of combinations of large and small tubes in series and in parallel. The bank model discussed in the next section provides both excellent separation capabilities and large flow rates.

### 3. ELUTION CHARACTERISTICS OF NETWORKS OF CAPILLARIES; APPLICATION TO GPC

Ideally, we would like to solve the problem of flow through an arbitrary combination of tubes of various diameters, widths and numbers. We have not been able to do this. But we have solved the problem for a more restricted class of networks which we have called "bank models". These models are useful in their own right as objects to be constructed, and also because they seem to be good models for GPC columns.

#### A. Description of the models

In this section we shall describe some models for a GPC column. A GPC column is made up of fine gel beads (*ca.*  $50 \mu$  in diameter) packed together. The beads are porous; we assume that the pores go through the entire bead. Thus we assume the carrier fluid flows around, into and through the beads.

The surface of the beads divides the system into two regions; that within the beads and that outside. In this picture of the column the region within the beads is viewed, for the purpose of calculation and simplicity, as made up of small open cylinders, all of radius  $r_s$ , and all of length  $l$ . We assume, naively, that each cylinder is of uniform radii and that no cylinder intersects another. These cylinders are assumed aligned in one direction and this direction is chosen as the direction of fluid flow. The number of small tubes per unit volume is chosen so that the total volume within small tubes is the same as that available to solvent within the beads. Furthermore these tubes must be bunched together so that the distance between bunches is comparable to the size of the interstitial region between beads in the real column. Thus both flow and diffusion are allowed within the beads as well as within the interstitial region between beads. Initially, none of the tubes will be closed. Thus there is no stationary or stagnant volume in our system. This restriction is easily removed.

The above model does not yet specify a detailed geometry for the system. We have purposely maintained generality because the method used to compute the broadening and skewness does not require a detailed specification. However in order to calculate average elution volumes as is done we have need of a more specific model. This latter model is a series of banks of tubes separated by mixing regions (see Fig. 2). We shall refer to this model as the bank model. Each bank is made up of parallel arrays of right circular cylinders of different radii; the fluid flows through the cylinders (rather than around them). For concreteness one can view each bank as a membrane riddled with holes. For clarity of presentation we assume there are tubes of only two radii,  $r_l$  and  $r_s$ ; their numbers in each bank are  $N_l$  and  $N_s$  and their length  $l$ . The



banks are thus all identical and there are  $n$  of them. This series of  $n$  banks is called a column.

The path of the particle through the column is as follows. A pressure head  $\Delta p$  forces the particle (which is suspended in carrier fluid) through the column from top to bottom. The particle emerges at time  $t$  later after having traversed  $np_l$  large tubes and  $np_s$  small ones. We assume that particles in the mixing region lose memory of the tubes they came out of. Accordingly the probability  $p_i$  of a particle jumping into and through the tubes of the next bank ( $i = l, s$ ) is independent of which tube it emerged from. This assumption will be more valid for larger diffusion coefficients and less valid for smaller diffusion coefficients. Its validity is also a function of the actual geometry of the system. For example, if all the large tubes were bunched together at one end of the bank (membrane) and the small tubes at the other end, one would expect that by piling the banks in register one could have large tubes in one bank vertically above large tubes in the adjacent banks. Consequently, particles coming from large tubes would tend to go into large tubes and so on. One obviously minimizes this effect by mixing the tubes within each bank.

The above bank model can be viewed in its own right as an object of study, or as a model for GPC. As a model for GPC, the large tubes represent the flow region outside of and around the beads; the small tubes represent pores in the beads. Any particle emerging from a small tube has the option of choosing to go into a small tube again (into a bead) or into a large tube (flow around a bead). Thus the totality of small tubes in one bank represents all the pores in all the beads at one level in the GPC column, and the totality of big tubes in this bank represents all the regions between beads at this same level. The length of the tubes is proportional to the diameter of the beads.

### B. The final formulae

The elution characteristics of the bank model have been calculated in our previous paper<sup>2</sup>. We have shown that the elution volume peak for a single species is Gaussian and have evaluated its first three moments. The procedure used is an adaptation of the method of HERMANS<sup>9</sup>. HERMANS allowed diffusion into and out of the beads while we have allowed both for flow through the beads and diffusion. Furthermore, we have evaluated the particle size dependence of the first moment (elution volume) in both the high fluid flow and low fluid flow (equilibrium) limits. One should consult ref. 2 for details. Here we will quote only the results.

$$\frac{\langle t \rangle}{x} = \left[ 1 + \frac{(1 - \phi)}{k\phi} \right] / \langle v_p \rangle_l' \quad (17)$$

$$\frac{\mu_2}{x} = \frac{l(1 - \phi)}{\phi k \langle v_p \rangle_s \langle v_p \rangle_l'} \mathcal{L} \left( \frac{l \langle v_p \rangle_s}{2D_s} \right) + \frac{2D_m}{\langle v_p \rangle_l'^3} \left[ 1 + \frac{(1 - \phi)}{k\phi} \right]^2 \quad (18)$$

$$\frac{\mu_3}{x} = \frac{-3(1 - \phi)l^2}{\phi k \langle v_p \rangle_s^2 \langle v_p \rangle_l'} \left[ \frac{1}{\left( \frac{l \langle v_p \rangle_s}{2D_s} \right)^2} - \frac{1}{\sinh^2 \left( \frac{l \langle v_p \rangle_s}{2D_s} \right)} - \frac{1}{3} \right] \quad (19)$$

$$+ \frac{6D_m}{\langle v_p \rangle_l'} \left( \frac{\mu_2}{x} \right) \left( \frac{\langle t \rangle}{x} \right)$$

$$\langle v_p \rangle_l' = \langle v_p \rangle_l \left[ 1 - \frac{(1 - \phi) \langle v_p \rangle_s}{\phi k \langle v_p \rangle_l} \right] \quad (20)$$

$$D_s = D_a + \frac{u_s^2 (1 - a/r_s)^6 r_s^2}{192 D_a} \quad (21)$$

$\mathcal{L}$  in eqn. (18) is the Langevin function,  $\mathcal{L}(x) = \coth(x) - 1/x$ . The length of the column is  $x$  and  $\langle t \rangle$  is the average time spent in it by the elutant.  $\phi$  is the fraction of flowing volume in large tubes,  $\langle v_p \rangle_l$  and  $\langle v_p \rangle_s$  are the average velocities in large and small tubes.  $\mu_2$  and  $\mu_3$  are the second and third moments about the mean for the time spent in the column.  $l$  is the length of the capillaries (in each bank) of which there are two types of radius  $r_s$  and  $r_l$  and the number of banks is, of course,  $x/l$ . The diffusion coefficient of the molecule of radius  $a$  is  $D_a$ . The maximum velocity of solvent in the small tube is  $u_s$  and an expression identical to eqn. 21 with  $m$  replacing  $s$  holds for  $D_m$ . The value of the quantity  $k$  depends on the flow rate through the system. In the limit of slow flow we have

$$\frac{1}{k} = K_e = (1 - a/r_s)^2 \quad a \leq r_s \quad (22)$$

$$K_e = 0 \quad a \geq r_s$$

For other shapes of tubes one replaces  $(1 - a/r_s)^2$  by the appropriate partition coefficient.

In the limit of fast flow one has

$$\frac{1}{k} = K_f = 1 / \left[ 2 - (1 - a/r_s)^2 - 2\gamma \left( \frac{a}{r_s} \right)^2 \right] \quad a \leq r_s \quad (23)$$

$$K_f = 0 \quad a \geq r_s$$

We have not been able to derive an expression for all flow rates, but an heuristic argument given previously suggests

$$\frac{1}{k} = K = K_f (1 - \exp(-u_s l / \beta D_a)) + K_e \exp(-u_s l / \beta D_a) \quad (24)$$

where  $\beta$  is a parameter near 1. The above moments (for the time it takes a particle to elute out of a column) are related to moments for the volume elution,  $V_e$ .

We obtain

$$\langle V_e \rangle = \bar{V}_e = g' V \left[ \phi + \frac{1 - \phi}{k} \right] \quad (25)$$

$$\frac{\langle (V_e - \bar{V}_e)^2 \rangle}{\bar{V}_e^2} = \frac{\mu_2}{\langle t \rangle^2} \quad (26)$$

$$\frac{\langle (V_e - \bar{V}_e)^3 \rangle}{\langle (V_e - \bar{V}_e)^2 \rangle^{3/2}} = \frac{\mu_3}{\mu_2^{3/2}} \quad (27)$$

$$g' = \frac{\left[ 1 + \frac{(1-\phi) u_s}{\phi u_l} \right]}{1 - \left[ \frac{(1-\phi) \langle v_p \rangle_s}{k\phi \langle v_p \rangle_l} \right]} \quad (28)$$

The quantity  $g'$  is always close to 1 for any system of interest.  $V$  on the right hand side of eqn. 25 is the total volume in small and large tubes. We have also shown that the elution volume peak is Gaussian.

### C. Discussion of results

In Fig. 5 we have plotted  $3 \log (a/r_s)$ , the logarithm of the cube of ratio of the particle radius to that of the small tube, *versus* the volume elution for various fractions of the volume available in the large tube,  $\phi$ , in both the flow limit and equilibrium limit. The variable  $(a/r_s)^3$  is proportional to the hydrodynamic volume of the polymer<sup>10</sup>.

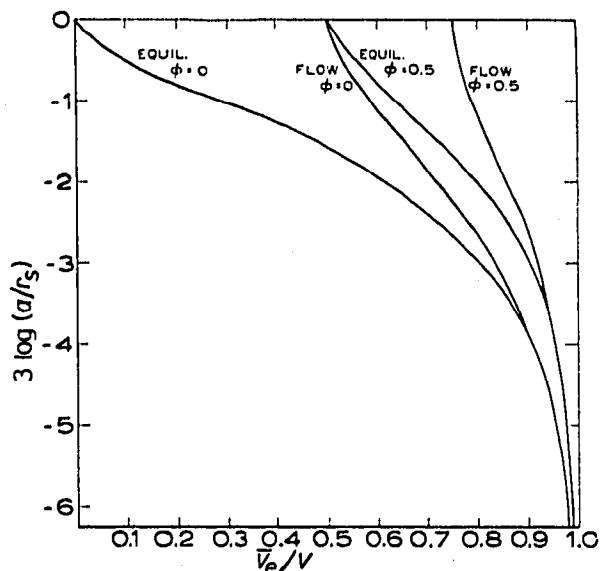


Fig. 5. Volume elution,  $V_e$ , *versus* log size (hydrodynamic volume) for the bank model for both the equilibrium and the flow limit for various values of  $\phi$  the fraction of volume in the interstitial region.  $V$  is total volume available to solvent.

The reader should notice that for small  $(a/r_s)$  the flow limit and the equilibrium limit are the same; one should also notice that the curves are similar in shape to experimental volume elution *versus* logarithm hydrodynamic volume curves of single column GPC systems. Clearly we could reproduce other experimental curves by suitable choice of a distribution of small tube sizes. (This statement is meant to suggest the sufficiency, not necessity, of the theory since others have produced such curves. Also see part D of this section.)

As we pointed out in Section 3 B, we have only derived the volume elution *versus* particle size equation in the limit of high flow and in that of low flow (equi-

librium). We also pointed out that from considerations of a single tube one might expect the more general volume elution equation as a function of flow rate  $V_e(u_s)$  to be

$$V_e(u_s) = V + KV_s \quad (29)$$

$$K = \theta K_e + (1 - \theta)K_f$$

$$\theta = \exp(-u_s l / \beta D_a)$$

In Fig. 6 we have plotted  $V_e - V_e(u_s = 0)$ , ( $\Delta V_e$ ), for various  $a/r_s$  as a function of  $u_s l / \beta D_a$  (we generally expect  $\beta \cong 1$ ). For large molecules  $u_s l / D_a$  is about 3 ( $u_s = (r_s/r_1)^2 u_1 = 10^{-4}$  cm/sec,  $l = 30$  microns,  $D_a = 10^{-7}$  cm<sup>2</sup>/sec) while for small molecules  $u_s l / D_a = 3 \times 10^{-2}$  (all parameters the same except  $D_a = 10^{-5}$  cm<sup>2</sup>/sec). Clearly except for large  $a/r_s$  we see very small changes in  $V_e$  as a function of flow except for a very high flow rate.

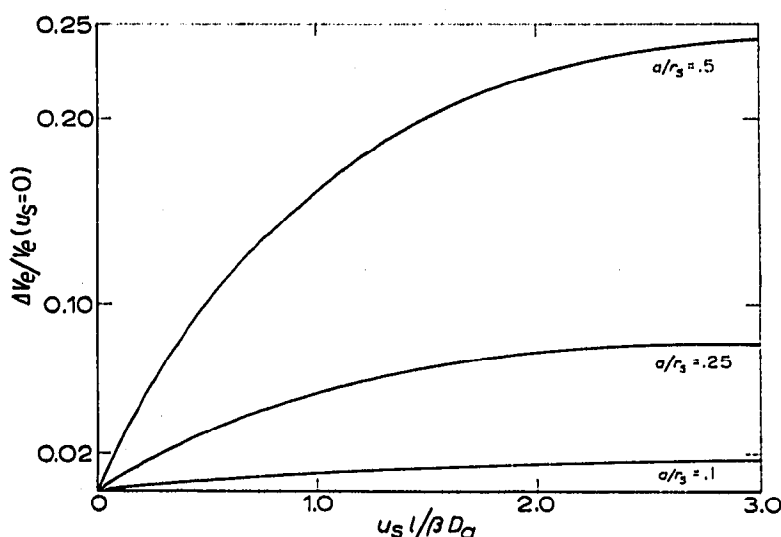


Fig. 6. Here we have plotted our estimate of the fractional change in volume elution as a function of  $u_s l / \beta D_a$ .  $\Delta V_e$  is the difference between the actual volume elution and that at zero flow ( $V_e(u_s = 0)$ ). For small  $a/r_s$  the change is minimal. These results are for a bank with only one size small tube;  $\beta$  is estimated to be one or two.

Our expression for the peak width eqn. 18 is similar to that derived by HERMANS<sup>9</sup> and indeed our expression includes the case of diffusion (diffusion only) as a limiting case. In Fig. 7 we have plotted the ratio of this expression to that obtained in the limit of zero flow. The ratio is always less than or equal to 1. This means that separation is always improved by using tubes with flow rather than tubes blocked up so that there is no flow.

The HETP (height equivalent theoretical plate) of a chromatographic column is normally defined as  $(V_e - \bar{V}_e)^2 / V_e$ , the dispersion in the volume elution divided by the volume elution. For small  $u_s l / D_a$  our expression for that part of the dispersion arising from the dispersion in small beads is similar to that obtained by others. For larger velocity and/or small diffusion coefficients the functional form changes dramatically. In Fig. 8 we have plotted the quantity  $(V_e - \bar{V}_e)^2 / [V_e(V_e - V_1)]$  versus  $u_s l / 2D_a$  for various ratios of bead size to small tube radius,  $l/r_s$ . The  $(V_e - V_1)$  in

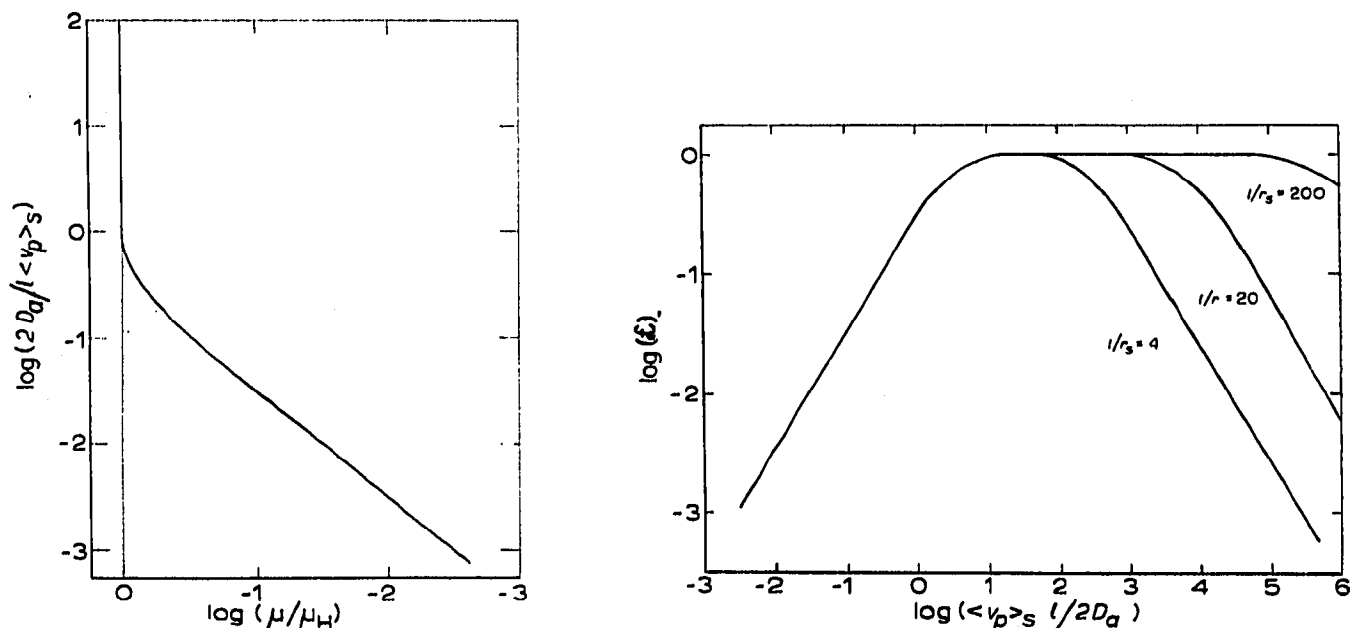


Fig. 7. Plot of the log of the ratio of second moment computed here to the second moment when no flow is allowed in the small tubes (the HERMANS limit)  $\mu_H$  as a function of the  $\log(2D_a/l\langle v_p \rangle_s)$  for  $l/r_s$  from 5 to 100. The quantity  $(2D_a/l\langle v_p \rangle_s)$  is essentially the ratio of the time for a particle to be flushed out of bead to the time it takes it to diffuse out. When that ratio is greater than one, the time to diffuse is smaller than the flush time. Our result goes to HERMANS' result. For this ratio less than one, the dispersion from our calculation is less than that from HERMANS'. (Notice this curve shows the ratio of  $\mu/\mu_H$  as a function only of  $(2D_a/l\langle v_p \rangle_s)$ . This is true for the range of  $(2D_a/l\langle v_p \rangle_s)$  we have chosen. For  $(2D_a/l\langle v_p \rangle_s) > 10^{-3}$  effects of changes in  $r_s$  are seen).

Fig. 8. Plot of logarithm of the Langevin function  $\mathcal{L}(\langle v_p \rangle_s l/2D_s)$  (where  $D_s = D_a + (u_s^2 r_s^2/192 D_a)$ ) versus  $\langle v_p \rangle_s l/2D_a$  for various  $l/r_s$ . The Langevin function is proportional to that part of the HETP arising from the dispersion inside the beads. Notice for small  $\langle v_p \rangle_s l/2D_a$  the curves are identical. This is the low velocity-high diffusion constant region and yields increasing dispersion as  $\langle v_p \rangle_s l/2D_a$  increases; in the high flow-small diffusion constant region, the dispersion decreases for increasing flow. The slopes of the shoulders of the curves are  $\pm 1$ .

the dispersion expression is the difference between the  $V_e$  and  $V_l$ , the volume elution of particles to big to go into the small tube. With the inclusion of this term in the expression for the dispersion, the only variable for a column of a single size of small tubes is  $\langle v \rangle_s l/2D_a$ . Thus we might expect that for a single column (except for some weak size dependence in  $\langle v_p \rangle_s/u_s$ ),  $u_s/D_a$  or, thus,  $u_l/D_a$ , the fluid velocity over the diffusion constant, is the important variable. One would expect the data for this part of the dispersion to scale that way.

The reader should notice that for small  $l\langle v_p \rangle_s/2D_a$ , the curves in Fig. 9 are identical. For larger  $l\langle v_p \rangle_s/2D_a$  they clearly break away from each other; for all curves however as  $l\langle v_p \rangle_s/2D_a$  gets above 1 the dispersion is either a constant or decreasing as  $\langle v_p \rangle_s/D_a$  increases. This is in contradistinction to the results obtained at smaller  $\langle v_p \rangle_s/D_a$ . Thus one might expect for a given column system that the dispersion for small molecules would increase for increasing  $u_l$  while that for large molecules (low diffusion constant) would decrease or remain constant for increasing  $u_s$ . Such results have been observed<sup>11</sup>.

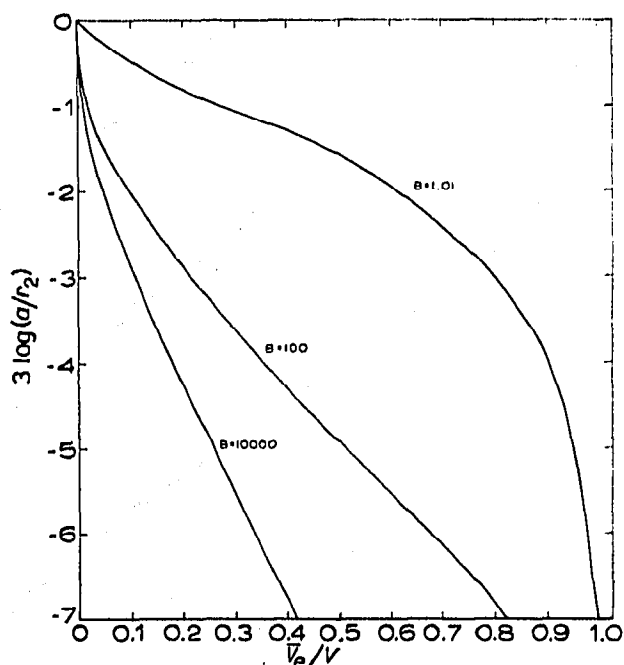


Fig. 9. A plot of the logarithm of the hydrodynamic volume *versus*  $\bar{V}_e/V$  for  $\phi = 0$  for a distribution of tube sizes (see eqn. 32). Notice as the ratio of the largest small tube to the smallest small tube,  $B$ , increases, the length of the linear portion of the curve increases, (For  $B = 10000$  the curve is linear from  $3 \log(a/r_2) = -3$  to  $3 \log(a/r_2) = -13$ ).

#### D. Application to distributions of tube sizes

Our equations are easily modified to include distributions of tube radii for the small tubes in each bank. Denoting the normalized distribution by  $g(r)$  so that  $V_s g(r) dr$  is the volume of cylinders of radius  $r$  within  $dr$  we have

$$\bar{V}_e = V_l + V_s \int_0^{\infty} g(r) K(a/r) dr = V_l + V_s \int_a^{\infty} g(r) K(a/r) dr \quad (30)$$

The use of  $a$  as the lower limit of integration is permissible because  $K(a/r) = 0$  for  $a > r$ . Eqn. 30 is valid if most of the flow is in the big tubes (interstitial region of the beads) which are not part of the distribution function  $g(r)$ . It is obvious from eqn. 30 that the shape of the volume elution curve is very much dependent on  $g(r)$  as well as  $K(a/r)$ . A choice of  $g(r)$  which makes the equilibrium volume elution curve linear as a function of the logarithm of the hydrodynamic volume is

$$g(r) = 1/[r \ln(B)] \quad r_1 \leq r \leq r_2 \quad (31) \\ = 0 \text{ elsewhere}$$

where

$$B = r_2/r_1$$

We obtain with use of eqn. 22,

$$\bar{V}_e = V_l + V_s \left[ \frac{-\ln(a/r_2)}{\ln B} - \frac{(3 - a/r_2)(1 - a/r_2)}{2 \ln B} \right] \quad r_1 \leq a \leq r_2 \quad (32)$$

$$\bar{V}_e = V_l + V_s \left( 1 - \frac{a}{\ln B} \left( \frac{1}{r_1} - \frac{1}{r_2} \right) \left[ 2 - \frac{a}{2} \left( \frac{1}{r_1} + \frac{1}{r_2} \right) \right] \right) \quad a \leq r_1$$

A plot of this function for various values of  $B$  is shown in Fig. 9. These curves have a much larger linear portion than those of Fig. 6. It is obvious from this example and the form of eqn. 30 that one can obtain a wide variety of elution volume curves by a judicious choice of  $g(r)$ .

There is an interesting insensitivity to the specific form of  $K(a/r)$  that occurs when  $g(r)$  is a very broad distribution function. This insensitivity is due to the step character of  $K(a/r)$  (that is, that for  $a > r$ ,  $K = 0$ , and for  $a \ll r$   $K = 1$ ). Thus if  $g(r)$  is so broad that most tubes have either  $r < a$  or  $r \gg a$  for all  $a$  then  $\bar{V}_e$  of eqn. 30 will not be sensibly changed by use of a step function in place of the correct functional form. For example, with a step function for  $K(a/r)$  and for the  $g(r)$  given by eqn. 31 we obtain

$$\begin{aligned}\bar{V}_e &= V_l & a &\geq r_2 \\ \bar{V}_e &= V_l + V_s \ln(r_2/a)/\ln(B) & r_1 &\leq a \leq r_2 \\ \bar{V}_e &= V_l + V_s & a &\leq r_1\end{aligned}\quad (33)$$

which is not much different from eqn. 32 over a wide range of values of  $a$ . To the extent that elution volume is insensitive to the difference between  $K$  and the step function  $\theta(a/r)$  we can say that the exclusion aspects of the formulation dominate.

#### *E. Generalization of the bank model to a combination of blocked and open capillaries*

If one allows a certain fraction of the small capillaries to be blocked up so that diffusion into and out of them is allowed but flow through them is disallowed then one obtains a more general model. In general one would expect that in GPC columns cavities of both types occur in the beads.

The average elution volume of such a column is identical in the equilibrium (slow flow) limit with that of the model in which there are only open tubes. Eqns. 17 and 25 remain valid with the substitutions afforded by eqn. 22 for  $K_e$ . Thus average elution volume is insensitive to whether there is flow through the capillaries.

Our equation for the second moment is now replaced by a sum over the two kinds of tubes. For the open tubes  $1 - \phi$  is replaced by the fraction of volume of tubes of the open type ( $\phi$  which is the fraction of volume of big tubes is unchanged). For the closed tubes  $1 - \phi$  is replaced by the fraction of volume of tubes of the closed type and also the Langevin is replaced by its argument. The effect of this replacement is to increase the broadening. This means that the elution characteristics are always improved by use of open-end pores rather than dead-end pores.

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