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Publisher *Taylor & Francis*

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Separation Science and Technology

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713708471>

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To cite this Article Giddings, J. Calvin and Brantley, Susan L.(1984) 'Shear Field-Flow Fractionation: Theoretical Basis of a New, Highly Selective Technique', *Separation Science and Technology*, 19: 10, 631 – 651

To link to this Article: DOI: 10.1080/01496398408060666

URL: <http://dx.doi.org/10.1080/01496398408060666>

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Shear Field-Flow Fractionation: Theoretical Basis of a New, Highly Selective Technique

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Abstract

Shear field-flow fractionation (shear FFF) is described as an FFF system in which shear forces are responsible for migration perpendicular to flow. It is shown that a desirable configuration for shear FFF is a concentric cylinder system with one cylinder rotating. After providing the relevant theoretical framework of FFF, the equations of Shafer et al. describing shear migration are simplified and applied to the limiting case of very thin annular spaces to get tractable retention expressions. On this basis the maximum selectivity is predicted to be 3 or greater, a value considerably higher than that for any other macromolecular separation technique. This high selectivity is confirmed using an alternate shear migration theory developed by Tirrell et al. However, it is shown that shear FFF is only applicable to macromolecules of high molecular weight, perhaps $\sim 10^7$ and above. It may also be applicable to globular particles.

INTRODUCTION

Field-flow fractionation (FFF) is a family of methods that has been developed rather extensively for the analytical scale separation of macromolecules and particles (1-5). FFF is an elution method like chromatography, but separation is achieved in the flowing carrier fluid without a stationary phase and without any obstructions (such as column packing) in the flow channel. The use of only one phase (the carrier fluid) is

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advantageous for macromolecules which often interact unfavorably and irreversibly at active interfaces between two partitioning phases.

The role of the stationary phase in chromatography—to induce differential retardation—is assumed by an external field or gradient. The external field must be one that interacts with the component particles, forcing them to migrate through the carrier fluid in the channel. However, the field is applied in a direction perpendicular to the flow, forcing the component particles toward one wall of the flow channel where they form a diffuse steady-state layer. Since the flow velocity approaches zero upon approaching the wall, the particles forced closest to the wall will be retarded to the greatest degree in their displacement by flow down the channel. Thus the separation occurs because different particle species are subject to different force levels from the external field, forcing them into layers near the wall of different mean thicknesses that are carried at unequal rates down the flow channel.

A number of fields and gradients have been proposed for use with FFF. Only four of these have resulted in experimental success. These four are electrical fields, thermal gradients, sedimentation fields, and flow or hydraulic forces. These have led to the FFF subtechniques termed electrical FFF, thermal FFF, sedimentation FFF, and flow FFF, respectively (3). [In addition, a limiting form of FFF termed steric FFF is applicable to larger particles, up to 100 μm in diameter (6).] The four subtechniques complement one another by virtue of different selectivities, different molecular weight ranges, and different levels of effectiveness which depend on the polarity, charge, density, etc., of the species of interest.

In this paper we present an initial evaluation of another subtechnique which we shall term shear FFF. This study indicates that shear FFF would have its own unique characteristics, both advantages and limitations, of such a nature that it might find a useful role in the stable of analytical separation techniques.

It is well recognized that gradients and shear forces will cause various species to migrate in a direction perpendicular to flow (7-9). Thus shear forces are obvious candidates to serve as the basis for constructing a new type of FFF system. The first suggestion for a shear FFF system appeared in one of the early disclosures of the FFF concept (10).

The shear-induced lateral migration can assume one of several apparent forms. In one form, flexible chain macromolecules tend to migrate through a shear gradient, in most flow situations seeking out regions of minimum shear (such as at the center of flow channels or stagnant pockets) where their configurational entropy is greatest. Such shear effects have recently been elucidated by Metzner (11). There are also shear forces originating in hydrodynamic effects, sometimes termed the "tubular pinch" effect. The latter has been proposed as the basis of a separation system (12). More

generally, shear migration is expected whenever nonhomogeneous flow is encountered (13, 14).

Clearly, one could attempt in a direct manner to apply these shear forces in FFF systems of more or less conventional design, consisting of simple flow channels or tubes (12) in which shear always accompanies flow. However, such an application would incorporate an immediate limitation on the normal versatility of FFF systems. In normal FFF systems the field strength and the flow rate can be controlled independently. This leads to substantial advantages, first in establishing the initial steady-state layers during a so-called stop-flow period, and following this the independent control and/or programming of field and flow to provide flexible optimization with respect to resolution, time, channel length, etc. However, if FFF were to utilize the normal gradient in shear rate generated in simple channel flow, then the force applicable to any species would be a function of the flow rate. In such an instance the force and the flow rate could not be controlled independently, leading to a loss of flexibility and perhaps serious conflicts in simultaneously achieving suitable flows and forces. (A slightly unbalanced oscillatory flow with a controllable net displacement rate might remove some of the above objections; relaxation to the steady-state could occur at zero net displacement.)

An analysis of the situation suggests that three active dimensions are preferred for a truly effective shear FFF system. The normal displacement flow must take place and be independently controllable along one axis. The migration of particles toward a wall would occur along a second axis. The flow responsible for shear effects would then occur along the third axis, perpendicular to the first to allow independent flow control, and perpendicular to the second in conformity with physical requirements.

The three-dimensional FFF system proposed above can be readily realized in the annular space between two concentric cylinders. The rotation of one cylinder induces a flow around the circumference of the device. This flow leads to shear forces with a direction perpendicular to the walls. The displacement flow can then occur independently along a third orthogonal coordinate directed along the common axis of the cylinders.

THEORETICAL BACKGROUND

Field-Flow Fractionation

The flow profile and the relative orientation of field and flow in FFF are indicated in Fig. 1. By convention, the field and flow vectors define the x and

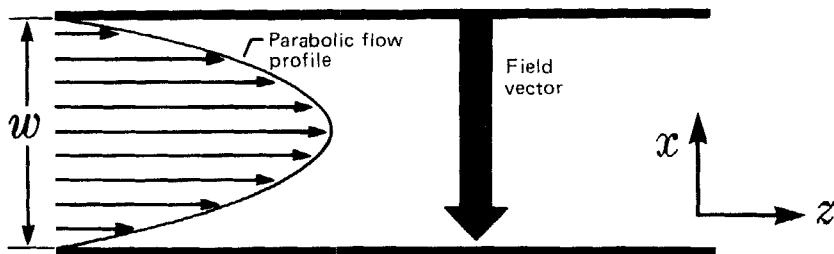


FIG. 1. Schematic illustration of flow profile in an FFF channel.

z axes, respectively. The field, acting on entrained particles, induces an average drift velocity U in the negative x direction (15, 16). The resulting concentration gradient produces a counteracting diffusional flux which soon balances the accumulation by drift, yielding a steady-state concentration profile of the form

$$c(x) = c_0 \exp\left(-\frac{x}{l}\right) \quad (1)$$

where c_0 is the concentration at the lower wall, $x = 0$, and l , a measure of the mean thickness of the particle layer, is

$$l = \frac{D}{|U|} = \frac{kT}{|F'|} \quad (2)$$

where D is the diffusion coefficient, k is Boltzmann's constant, T is temperature, and F' is the force acting to displace each particle.

The dimensionless retention parameter λ is defined as the ratio of layer thickness l to column thickness w :

$$\lambda = \frac{l}{w} = \frac{D}{|U|w} = \frac{kT}{|F'|w} \quad (3)$$

The critical quantity in an FFF separation device is the retention ratio R of a particular zone, calculated as

$$R = \frac{\mathcal{V}}{\langle v \rangle} = \frac{\langle c(x) \cdot v(x) \rangle}{\langle c(x) \rangle \langle v(x) \rangle} \quad (4)$$

where v is the carrier flow velocity in axial direction z , \mathcal{V} is the mean particle velocity, $\langle v \rangle \equiv \langle v(x) \rangle$ is the cross-sectional average carrier velocity, and $\langle c(x) \rangle$ is the cross-sectional average concentration. When these averages are expressed as integrals and solved explicitly for flow between infinite parallel plates, R is found to be related as follows to λ (16):

$$R = 6\lambda[\coth(2\lambda)^{-1} - 2\lambda] \quad (5)$$

As one approaches the generally desirable limit of small λ , this becomes

$$R = 6\lambda \quad (6)$$

Steric FFF

The above treatment assumes that particles can occupy the entire space between two parallel walls. When particle radius a is a significant fraction of l , this assumption is no longer valid because the centers of gravity of particles are excluded by the particles' own size from a thin layer near the walls. This is termed a *steric effect*. The steric effect perturbs the distribution of particles with respect to the wall, essentially pushing the particles further from the wall and thus increasing the average particle velocity and the resulting retention ratio R . The increment in R can be described approximately by adding a steric term to Eq. (6), giving (17)

$$R = 6\lambda + 6\gamma\alpha = 6\frac{l}{w} + 6\gamma\frac{a}{w} \quad (7)$$

where γ is a flow-dependent term of order unity and $\alpha = a/w$.

When the particles are sufficiently large and l is sufficiently small that $a \gg l$, Eq. (7) is dominated by the steric term, giving

$$R = 6\gamma a/w \quad (8)$$

Under these circumstances, the FFF method is termed *steric FFF*. The transition from normal to steric FFF is interesting but complicated because with normal FFF (controlled by the first term on the right-hand side of Eq. 7) small particles generally migrate faster and elute ahead of large particles whereas with steric FFF the elution order is inverted (18).

Selectivity

The efficacy of a macromolecular separation, whether attempted by normal FFF, steric FFF, or size exclusion chromatography, can be related to column efficiency and selectivity. Column efficiency, an index of the narrowness of component zones, is measured by theoretical plate height and the number of theoretical plates. For both FFF and chromatography, column efficiency varies over wide limits depending upon conditions.

Column selectivity is a measure of the column's intrinsic ability to disengage component zones (18, 19). While selectivity can vary somewhat, there tends to be a maximum approachable value which clearly spells out the fractionating power of a column.

Specifically, the *mass selectivity* is defined by (18)

$$S = \left| \frac{d \log V_r}{d \log M} \right| = \left| \frac{d \log R}{d \log M} \right| \quad (8a)$$

where M is the molecular weight and V_r is the retention volume. The maximum selectivity value for normal FFF can be shown to approach the limit (19)

$$S_{\max} = \left| \frac{d \log \lambda}{d \log M} \right| \quad (8b)$$

Values of S_{\max} range from unity for sedimentation FFF to about 0.5 for thermal and flow FFF and 0.33 for steric FFF. For size exclusion chromatography, the values are considerably lower: 0.05 to 0.22 (19). The difficulty (as measured by the required number of theoretical plates) of achieving a separation increases with the inverse square of S_{\max} . Consequently, the above differences are of great practical importance.

SHEAR FFF

With any new FFF subtechnique such as shear FFF, one of the principal criteria of effectiveness is the above defined mass selectivity. In the following theoretical development of shear FFF, we will obtain expressions for the dependence of λ on M , thus allowing an evaluation of S . We will find that S is potentially higher than that for any of the techniques so far discussed, approaching a maximum value of 3.

Normally, FFF (normal or steric) is carried out in a ribbon-shaped channel. However, the annular space between two concentric cylinders may have special merit in some forms of FFF; among other things, the flow in such a channel will avoid the disturbance of edge effects.

In the proposed shear FFF apparatus, the channel will of necessity consist of the annular space between concentric cylinders to accommodate relative rotation. With an inner cylinder of radius R_1 and an outer cylinder of radius R_2 , the column thickness w will simply be $R_2 - R_1$ (see Fig. 2). If $R_1 \gg w$, the annular channel will behave in most respects like an infinite parallel plate channel of gap thickness w . Rotation of one cylinder at the angular velocity of Ω rad/s will create the shear force and particle drift necessary for separation. Such circular Couette flow is described in standard textbooks (20). Carrier flow in the axial direction is superposed over this angular flow. Studies (21, 22) have shown that for a Newtonian fluid, the shear and channel flows are indeed superposable. Tanner found that the pressure drop along the axial direction was not distorted by the shearing flow created by relative rotation.

According to Shafer, the curvilinearity of the shear flow causes the inward migration of polymer molecules. Shafer assumed a dilute solution of noninteracting polymer molecules. The derivation was based on the Zimm bead-spring model for large, random-coil polymers (23). The model entails $N + 1$ identical beads connected by N segments, which are mechanistically equivalent to N Hookean springs, each with force constant $3kT/b^2$, where b is the root-mean-square length of a spring, k is Boltzmann's constant, and T is the absolute temperature. The model assumes that only the beads interact

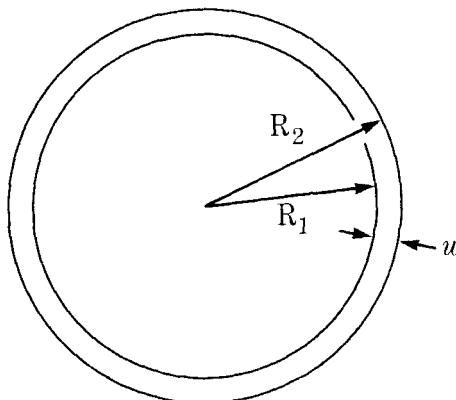


FIG. 2. End view of shear FFF channel consisting of concentric cylinders with radii R_1 and R_2 . The gap between cylinders is the FFF channel.

with the solvent. The amount of hydrodynamic interaction is indicated by the value of h , the draining parameter. The variable h is a measure of the freedom of fluid flow through the segmental polymer molecule

$$h = \frac{N^{1/2} f_{\text{bead}}}{(12\pi^3)^{1/2} b \eta} \quad (9)$$

where f_{bead} is the friction coefficient of one bead and η is the carrier viscosity.

Zimm theory defines two limiting cases. In the limit of $h = 0$, the free-draining case, there is no hydrodynamic interaction between polymer segments. This free-draining case refers to a polymer which does not perturb the fluid velocity field. In the limit $h \gg 1$, the nondraining case, there is a very large hydrodynamic interaction between segments. In this case the carrier velocity at the center of the polymer chain is identical to the polymer velocity at that point. The polymer chains then behave like rigid-sphere molecules (24).

Shafer predicted that the inward migration velocity for the two cases would be

$$U_{\text{non}} = \frac{-0.00845 \eta L^5}{kT} \left(\frac{4\Omega^2 R_1^4 R_2^4}{(R_2^2 - R_1^2)^2 r^5} \right) \quad (10)$$

$$U_{\text{free}} = \frac{-0.000617 f_{\text{bead}} b^4 N^3}{kT} \left(\frac{4\Omega^2 R_1^4 R_2^4}{(R_2^2 - R_1^2)^2 r^5} \right) \quad (11)$$

where L is the root mean square end-to-end length of the polymer molecule and r is the distance along the radial coordinate. The coefficients 0.00845 and 0.000617 arise from analytical integration.

We note that the parenthetical expressions in Eqs. (10) and (11) are related to the shear rate $K(r)$ by

$$\frac{4\Omega^2 R_1^4 R_2^4}{(R_2^2 - R_1^2)^2 r^5} = \frac{K^2(r)}{r} \quad (12)$$

where $K(r)$ is given by

$$K(r) = \frac{2\Omega R_1^2 R_2^2}{(R_2^2 - R_1^2)r^2} \quad (13)$$

Clearly the shear rate $K(r)$ increases as r approaches R_1 . The inward (i.e., negative) velocity predicted by Eqs. (10) and (11) indicates that the macromolecules tend to move toward this region of increased shear strength. High molecular weight polymers will thus move toward the inner cylinder.

In the limit of small w , a simpler expression for $K^2(r)/r$ is possible. Let

$$\Delta = w/R_1, \quad \Delta \ll 1 \quad (14)$$

Then

$$R_2 = R_1(1 + \Delta) \quad (15)$$

and

$$\frac{R_2^4}{(R_2^2 - R_1^2)^2} = \frac{1 + 4\Delta + 6\Delta^2 + 4\Delta^3 + \Delta^4}{4\Delta^2 + 4\Delta^3 + \Delta^4} = \frac{1}{4\Delta^2} \left(1 + 3\Delta + \frac{11}{4}\Delta^2 + \frac{1}{2}\Delta^3 - \frac{3}{16}\Delta^4 + \dots \right) \quad (16)$$

which, to a first approximation, yields

$$\frac{R_2^4}{(R_2^2 - R_1^2)^2} = \frac{1}{4\Delta^2} \quad (17)$$

This approximation will entail a fractional error of $\sim 3\Delta$ which, for example, given a Δ of 0.01, is of the order of 3% error.

Similarly, to make the term r^{-5} more tractable, we define δ by

$$r = R_1(1 + \delta) \quad (18)$$

where $\delta \leq \Delta$ and thus $\delta \ll 1$. Substitution of this into r^{-5} and a Taylor expansion yields

$$\frac{1}{r^5} = \frac{1}{R_1^5} (1 - 5\delta + 15\delta^2 - 35\delta^3 + 70\delta^4 - \dots) \quad (19)$$

The coordinate range of interest in practical FFF is a narrow range (a few l 's in thickness) near the wall at R_1 where particle accumulation takes place.

Thus δ , far more than Δ , can be neglected by comparison to unity. Consequently, the last equation can be approximated by

$$\frac{1}{r^5} = \frac{1}{R_1^5} \quad (20)$$

With the use of Eqs. (17), (20), and (14), the expression of Eq. (12) becomes

$$\frac{4\Omega^2 R_1^4 R_2^4}{(R_2^2 - R_1^2)^2 r^5} = \frac{\Omega^2}{R_1 \Delta^2} = \frac{\Omega^2 R_1}{w^2} \quad (21)$$

When this is substituted into Eqs. (10) and (11) and the absolute values of the latter are used in Eq. (3), we get the retention parameter λ for the nondraining case

$$\lambda_{\text{non}} = \frac{D w k T}{0.00845 \eta L^5 \Omega^2 R_1} \quad (22)$$

and for the free draining case

$$\lambda_{\text{free}} = \frac{D w k T}{0.000617 f_{\text{bead}} b^4 N^3 \Omega^2 R_1} \quad (23)$$

In order to calculate the magnitude of these λ values (and thus of FFF retention) for specific polymers, we must estimate the polymer parameters and relate them to polymer molecular weight.

In the Zimm model used to derive the lateral migration velocities, the root-mean-square end-to-end distance L of the molecule is calculated as an average of the end-to-end distance vector over the phase space distribution function of the model (25). As long as N is large and the end-to-end distance is smaller than about $0.5(N + 1)b$, the distribution function of a completely freely rotating chain can be approximated by a random flight distribution. This yields

$$L = N^{1/2} b \quad (24)$$

However, real polymers' dimensions will differ from this prediction, partly because the freely jointed chain model does not take into account the physical impossibility of two beads occupying the same position. Due to this excluded volume effect, the average dimension of the chain will be somewhat

larger than that predicted by Eq. (24). A correction factor, the molecular expansion coefficient α , is used to provide a better approximation (25). Parameter α is a function of the polymer, the solvent, and the temperature. Although the excluded volume effect predicts an α greater than unity, a poor solvent can often be found such that α for a polymer is equal to 1. Such a solvent is called a θ -solvent and the temperature at which α equals 1 is called the θ -temperature. Equation (24) is applicable in a rigorous sense only to θ -solvents; it is an approximation for other solvents.

In the Zimm model, the molecular weight M of the polymer is simply the summation of the contribution of the identical beads, each of mass m :

$$M = (N + 1)m \cong Nm \quad (25)$$

The molecular weight dependence of L from Eq. (24) then is

$$L = M^{1/2}(b^2/m)^{1/2} = M^{1/2}B \quad (26)$$

where B is a constant for a given polymer type. The theoretical prediction that L is proportional to the square root of the molecular weight holds rigorously, of course, only at the θ -temperature of any particular solvent.

The approximate ratio B of end-to-end distance to the square root of the molecular weight is needed in order to correlate the molecular weight of a polymer to FFF retention for the nondraining model. This parameter can be determined experimentally. Values for various polymers and solvents are listed in standard tables (26).

Using B for the experimental ratio $L/M^{1/2}$, the retention parameter-molecular weight relationship for the nondraining case is derived from Eq. (22)

$$\lambda_{\text{non}} = \frac{D w k T}{0.00845 \eta B^5 M^{5/2} \Omega^2 R_1} \quad (27)$$

The dependence of the diffusion coefficient D on molecular weight should also be considered. We start with Einstein's equation (24)

$$D = kT/f \quad (28)$$

According to the Zimm model, the friction coefficient for the nondraining polymer can be expressed by

$$f_{\text{non}} = L \eta / 0.192 \quad (29)$$

The diffusion coefficient can then be written as

$$D = 0.192kT/L\eta = 0.192kT/BM^{1/2}\eta \quad (30)$$

which can be inserted into Eq. (27) to give

$$\lambda_{\text{non}} = \frac{w(kT)^2}{0.0440\eta^2 B^6 M^3 \Omega^2 R_1} \quad (31)$$

This expression may incur a numerical error since there is some doubt as to whether Eq. (29) is applicable in this particular situation of shear flow (7). Equation (31) does, however, give an approximate indication of the molecular weight dependence which is expressed in the third power term, M^3 . This indicates that λ is very sensitive to M and that the separation process is potentially very selective (see below).

For the free-draining case, the frictional coefficient for the entire molecule is simply the sum of the individual frictional coefficients of the N beads (8):

$$f = Nf_{\text{bead}} \quad (32)$$

With this equation and Eqs. (25), (26), and (28), Eq. (23) becomes

$$\lambda_{\text{free}} = \frac{w(kT)^2 m^2}{0.000617 f_{\text{bead}}^2 B^4 M^4 \Omega^2 R_1} \quad (33)$$

This equation shows a fourth-power dependence of λ on molecular weight, suggesting an even more selective separation than implied by the nondraining model.

For both nondraining and free-drawing models applied to shear FFF, and for most other FFF subtechniques as well, λ is a simple inverse power function of M :

$$\lambda = \text{const}/M^s \quad (34)$$

Application of Eq. (8b) shows that

$$S_{\text{max}} = s \quad (35)$$

We have just shown that the exponent s is 3 and 4 for nondraining and free-draining models, respectively. Consequently, the respective maximum

selectivity calculated in accordance with these models is 3 and 4. Both values are extremely high, but it is important to know, specifically, which one is applicable in practice.

Theoretical treatments (27-29) dealing with the dependence of various polymer properties on h indicate that there is no completely satisfactory method available to approximate the behavior of polymers in which the hydrodynamic interaction is intermediate between the free-draining and nondraining cases. In general, the nondraining limit seems to apply to real polymer solutions, especially those systems approaching the limit of infinite dilution (24). Equations (27) and (31) can thus be surmised as the expressions most applicable to an FFF system. We are thus led to the lesser of the two proposed maximum selectivity values

$$S_{\max} \cong 3 \quad (36)$$

The prospect that the mass selectivity of a shear FFF column might be in the vicinity of 3 is rather exciting. As noted earlier, the selectivity of other currently available polymer separation techniques is, at most, unity, the S_{\max} value calculated for sedimentation FFF (18, 19). Selectivity for exclusion methods of chromatography (gel filtration and gel permeation chromatography) are 5-20 times lower still, some 15- to 60-fold below that calculated for shear FFF. A tripling in selectivity means that a shear FFF column can be 1/9th the length and would need 1/9th the elution time necessary for a sedimentation FFF column yielding results of comparable plate height. The shear FFF column would need to have less than 1% of the theoretical plates of an exclusion column for equivalent resolution.

ALTERNATE THEORY

The above treatment is based on the Zimm model of macromolecules, which assumes a dilute solution of noninteracting chains, and upon the Shafer theory of cross-migration. Departures from theory may arise because underlying conditions are not fulfilled, or because the theoretical models are not complete. In the former category we expect some difficulty with the buildup of polymer at the inner cylinder, which may lead to concentrations in excess of those appropriate for the Zimm model, and could cause significantly increased viscosity and result in flow perturbations. Shafer noted that although such effects would be generally negligible when working with molecular weights of less than 10^6 , molecules the size of chromosomal DNA might involve significant effects.

In the latter category we note that Shafer assumes in his derivation that the shear rate $K(r)$ is constant over molecular dimensions so that the Couette flow distribution function can be replaced with the simple shear laminar flow distribution function. It is this approximation that Aubert and Tirrell claim to improve substantially in their treatment (13).

The Aubert and Tirrell treatment of cross-streamline polymer migration in Couette flow uses two models: the Rouse model which is a bead-spring polymer model substantially similar to the Zimm model with $h = 0$ (30), and an elastic dumbbell model (25). However, Aubert and Tirrell derive a cross-migration velocity only for the elastic dumbbell model since they do not find it possible to derive the configuration space distribution function of bead positions for a Rouse model polymer in nonhomogeneous flow. Aubert and Tirrell find that, for the dumbbell, Shafer's approximation of constant shear rate over molecular coordinates is applicable. The derivation under this second treatment is not therefore a basic improvement upon Shafer's original treatment, but rather is valuable because it offers results for a complementary model.

Following procedures much like those above, the results can be shown to yield

$$\lambda_{db} = \frac{4wDH'}{\tau kT\Omega^2 R_1} \quad (37)$$

This prediction of λ , as noted, is based on the elastic dumbbell model which consists of two beads connected by a spring with force constant H' and relaxation constant τ . We use the expression (13, 9)

$$\tau = f_{\text{bead}}/4H' \quad (38)$$

and, as before (25),

$$H' = 3kT/b^2 \quad (39)$$

where f_{bead} and b are defined as previously for a freely jointed chain model. With these substitutions we get

$$\lambda_{db} = \frac{144DwkT}{f_{\text{bead}}b^4\Omega^2 R_1} \quad (40)$$

which resembles Eq. (23). The friction coefficient of the dumbbell, assuming

free-draining, is again the summation of the individual friction coefficients of the identical beads (25), $f = 2f_{\text{bead}}$, giving

$$f_{\text{bead}} = f/2 \quad (41)$$

Using this and Eq. (28), both f_{bead} and D can be expressed in terms of f , giving

$$\lambda_{db} = \frac{288w(kT)^2}{f^2 b^4 \Omega^2 R_1} \quad (42)$$

If we can determine the molecular weight dependence of f and b , the molecular weight dependence of λ_{db} will emerge for comparison with the λ —molecular weight relationships of the last section.

We note that for the extended bead-spring models, increasing molecular weight is represented by an increasing number of beads and springs. For the dumbbell model, increasing molecular weight must be envisioned as equivalent to the physical growth of the dumbbell. Since the physical dimension L ideally increases with $M^{1/2}$, we must imagine the bead-to-bead distance in the dumbbell to do likewise, giving us an expression parallel to Eq. (26):

$$b = B_{db} M^{1/2} \quad (43)$$

The friction coefficient must also increase with M in order to represent the increasing drag of increasingly bulky molecules. If we assume, following Stokes' law, that f is proportional to viscosity η and to some characteristic dimension of the particle (radius or diameter for spheres; b for dumbbells), we have

$$f = \eta B'_{db} M^{1/2} \quad (44)$$

The substitution of Eqs. (43) and (44) into Eq. (42) yields

$$\lambda_{db} = \frac{288w(kT)^2}{B_{db}^6 M^3 \eta^2 \Omega^2 R_1} \quad (45)$$

where constant $B_{db}^6 = B'^2_{db} B'^4_{db}$. This λ has exactly the same form as Eq. (31), a fact demonstrating the consistency of the two treatments and reinforcing

the expectation that the mass selectivity S will approach 3 for the shear FFF system.

Although the above equations are highly approximate, we can use them to estimate the molecular weight limits for which practical retention levels are expected. If, for example, Eq. (31) is applied to an aqueous system ($\eta = 10^{-2}$ poises) at room temperature containing a typical polymer with (26) $B = 10^{-8}$ cm/(g/mol), we get

$$\lambda \sim \frac{20}{\Omega^2(M/10^8)^3} \sim \frac{2000}{(\text{rpm})^2(M/10^8)^3} \quad (46)$$

In any FFF system, practical retention occurs at $R \leq 0.5$, corresponding roughly to $\lambda \leq 0.1$ (31). Thus angular velocities greater than about 15 rad/s (approximately 150 rpm) show promise for separating macromolecules with molecular weights on the order of 10^8 and above. Roughly 4500 rpm would be required for $M = 10^7$. However, these values are highly approximate, partly because of the high sensitivity of λ to changes in B (sixth power); the migration data of Shafer et al. suggest even stronger retention (lower λ 's).

EXTENSIONS AND LIMITATIONS

It is likely that shear FFF could also be used to fractionate globular particles. The phenomenon of cross-streamline migration of small suspended particles in shear flow has been studied ever since the investigations by Segré and Silberberg (32, 33). Halow and Wills (34) investigated the cross-streamline migration of spherical particles in circular Couette flow. They found that neutrally buoyant spheres were carried to an equilibrium position near the midpoint of the annular space, slightly closer to the inner than the outer wall. In subsequent theoretical work, Halow and Wills (35) concluded that the radial migration was caused by the drag exerted on the sphere suspended in shear flow. However, they found it necessary to use an empirical multiplier of 5 to correlate their theoretical predictions with experimental findings.

Ho and Leal (36) developed a more extensive theory for the inertial migration of rigid spheres in flowing suspensions. This theory needs no empirical factors. They concluded that in simple shear flow, rigid spheres will migrate to an equilibrium position halfway between the two confining walls. In Couette flow, there is a tendency to migrate closer to the inner wall. For an annular gap thickness w which is small compared to the cylinder radii, particles in Couette flow could be expected to behave as if they were

undergoing simple shear flow. Ho and Leal derived the following equation expressing the radial force exerted on the neutrally buoyant particles due to inertial effects

$$F = -\rho(\Omega R_1)^2 \frac{a^4}{w^2} G_1 \quad (47)$$

where ρ is the solvent density, a is the particle radius, and G_1 is a function of the particle distance from the wall. Ho and Leal gave values of G_1 for various particle positions. Since G_1 changes sign at $x = w/2$, the theory predicts that any neutrally buoyant particle will migrate to the centerline of the annular space.

Ho and Leal did not deal explicitly with nonneutrally buoyant particles. Halow and Wills derived an equation which indicates that nonneutrally buoyant particles migrate toward different equilibrium positions. This suggests that a shear FFF device might be used to separate particles of differing densities. More experimental work and theoretical analysis is clearly necessary to clarify this possibility.

The more recent work of Aubert, Prager, and Tirrell (14) indicates that cross-flow migration has the same origins for flexible chain macromolecules as for rigid particles. Based on a comparison with work reported by Brenner (37), particle size, not flexibility, appears to play a critical role in such migration processes. Thus rigid particles of colloidal and larger dimensions are likely subject to fractionation by shear FFF.

All the evidence we have cited suggests that shear forces are negligible for small molecules and even for polymers of average dimensions. In all likelihood, therefore, most of the particles to which shear FFF will be applicable will have large effective diameters, probably 1 μm or greater. For such large particles, steric forces are almost always important. In some cases they are dominant, leading to steric FFF. There is, of course, no inherent difficulty in operating shear FFF in the steric mode. However, the selectivity in the steric mode (~ 0.33 for globular particles, 0.50 for random chain polymers) is far less than that expected (~ 3.0) for the normal form of shear FFF, a value we have discussed earlier. Also, one must be careful to avoid the transition region between steric and normal FFF because the two modes of operation tend to nullify one another by virtue of the inversion of elution orders (18).

An interesting consideration in the steric FFF (induced by shear, sedimentation, or other fields) of large random-coil macromolecules such as DNA is the elasticity of the random coil. It can be presumed that the random coil would show some compression when forced against a wall by the lateral force, thus reducing the effective profile height of the random-coil molecule.

This in turn would reduce the migration rate in the steric FFF mode. The likelihood of a significant compressional displacement is supported by the fact that the energy $F'a$ of displacement of the random coil over its radius a is approximately a/l times thermal energy kT , as shown by Eq. (2). (Displacement over a would correspond to the energy expended in partially flattening the random coil.) Since the ratio $a/l \gg 1$ in steric FFF, compressional energies will exceed kT and deformation should therefore be significant.

In any experimental implementation of shear FFF, stable laminar flow is desired. Turbulence can be avoided by using low rates of shear. Instabilities are avoided by rotating the outer rather than the inner cylinder. Criteria are provided by Landau and Lifshitz (38). For example, if the inner cylinder is rotated, which may be an experimental advantage, instability is reached at angular speeds described by Landau and Lifshitz as

$$\Omega = 41.3 \eta / \rho w \sqrt{w R_2} \quad (48)$$

Thus in an experimental system with $R_1 = 0.475$ cm, $R_2 = 0.500$ cm, and $w = 0.025$ cm, the limiting angular velocity would be on the order of 145 rad/s, or roughly 1400 rpm. Much higher rotation rates would be possible with rotation of the outer cylinder.

High angular velocities may cause centrifugal effects of sufficient magnitude to influence retention. The centrifugal force on a particle rotating around an axis can be expressed as (39)

$$F'_c = (M/N_a)(\Delta\rho/\rho_s)\omega^2 r \quad (49)$$

where M is the molecular weight of the particle, N_a is Avogadro's number, $\Delta\rho$ is the difference between carrier density and particle density, ρ_s is the particle density, and ω is the local angular velocity. The combination of Eqs. (3) and (27) yields the competing shear force

$$|F'| = \frac{0.00845 \eta B^5 M^{5/2} \Omega^2 R_1}{Dw^2} \quad (50)$$

The relative magnitude of the disturbance is therefore

$$\frac{F'_c}{|F'|} = \frac{Dw^2(\Delta\rho/\rho_s)(r/R_1)(\omega/\Omega)^2}{0.00845 \eta B^5 M^{3/2} N} \quad (51)$$

which depends rather strongly on molecular weight, viscosity, density differences, etc. The ratio should be greater for globular particles than

polymers because of the generally higher densities. The disturbance due to F'_c can be reduced by rotating the outer cylinder, in which case $\omega \cong 0$ near the inner cylinder where the particles accumulate. However, if $F'_c > |F'|$ at the outer wall, there would be no mechanism for transferring particles from the vicinity of the outer wall to the exponential layer near the inner wall so that the process could begin.

We note also that shear FFF, like other forms of FFF, is subject to overloading effects when sample size is too great. The effects may generally be more serious for shear FFF because of the larger molecules employed. The latter molecules may also show unusual effects because of their susceptibility to distortion by shear. At sufficiently (unacceptably) high shears, chain rupture may also occur. The critical rotation rate for the shear degradation of various molecular weights of one water-soluble polymer (polyacrylamide) in Couette flow has been determined by Abdel-Alim and Hamielec (40).

Another possible disturbing factor in shear FFF is the heat released during viscous shear and the development of temperature gradients to dissipate that heat. Substantial gradients would be a source of convection and other perturbations.

The temperature drop across the annular gap can be estimated using the equation (41)

$$\Delta T = \frac{\eta(K(r))^2 w^2}{2\kappa} \quad (52)$$

where η is the solvent viscosity and κ is the thermal conductivity of the solution. This equation assumes that one cylinder wall is a heat barrier, which is the worst possible case. Assuming that $w \cong 0.025$ cm, $R_1 \cong 0.50$ cm, $\eta \cong 0.01$ g s⁻¹ cm⁻¹, and $\kappa \cong 6 \times 10^4$ erg/cm · s · K (values for water at 20°C), the calculated temperature drop is on the order of $10^{-8} \times \Omega^2$. With angular velocities as high as 200 rad/s ($\cong 2000$ rpm), this still creates thermal increments on the order of only 0.0004°C.

The problem associated with the additional drag caused by liquid at the ends of the cylinder has been estimated in other experiments in which annular column lengths were varied (42). According to Merrington (43), a cylinder 100 times as long as the width of the annular space will exhibit negligible end effects. A small annular space is also necessary in order to guarantee a constant shear rate across the column.

When first injected into the shear FFF system, macromolecules are distributed over the entire cross-section of the channel at the injection site. A finite time is necessary for the molecules to "relax" into the steady-state

exponential layer described by Eq. (1). To avoid zone distortions, flow must be halted immediately after injection for a time adequate to achieve the exponential distribution. It has been shown (16) that a simple expression for an adequate stop-flow time in an FFF system is

$$t_{\text{stop}} = w/U = w^2 \lambda/D \quad (53)$$

which is just the time necessary to travel across the thickness w of the channel at the induced field velocity U . For shear FFF, the necessary stop-flow time can be expressed by substituting Eq. (27) into the above

$$t_{\text{stop}} = \frac{w^3 k T}{0.00845 \eta B^5 M^{5/2} \Omega^2 R_1} \quad (54)$$

This equation shows that high molecular weight polymers require a considerably shorter period of time to achieve steady-state conditions than lower molecular weight polymers. For a polymer of molecular weight 10^8 and an angular velocity of 100 rad/s in a water-filled shear FFF channel with a w of 0.025 cm and radius of 0.50 cm, the relaxation time would be approximately 3 min, which is an acceptable delay.

CONCLUSIONS

Our theoretical analysis suggests that a functional shear FFF system would yield the highest selectivity known for the fractionation of polymers of high molecular weight. The method is likely applicable to globular particles as well. However, the shear effect is so intrinsically weak that only those polymers of molecular weight $\sim 10^7$ or greater are likely to be resolved. A good deal of experimental work is clearly needed to fully delineate the potential of shear FFF.

Acknowledgment

This material is based upon work supported by the National Science Foundation under Grant No. CHE-8218503.

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