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FIELD FLOW FRACTIONATION

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I. INTRODUCTION

Field-Flow Fractionation (FFF) is a relatively new technique with considerable potential in the field of macromolecular separations¹⁻⁵. Conventional separation tools, such as gas chromatography (GC), extraction, and liquid chromatography (LC), frequently fail in the case of macromolecules for several reasons. The low vapor pressure of macromolecules rules out GC, at least at low pressures. Classwise chemical similarity and the tendency of macromolecules to exhibit extremes in phase distribution make LC and extraction methods difficult⁶. The separation methods that have found the greatest use in the field of large molecules are gel filtration, ion-exchange chromatography, electrophoresis and ultracentrifugation (with or without gradients). Some of these techniques are limited in peak capacity. In the case of gel filtration, for instance, definite upper and lower retention limits exist, and the peak capacity is correspondingly reduced⁷. Peak capacities in electrophoresis and ultracentrifugation have a square-root dependence upon the applied field^{8,9}. A cascading of the separating power of either of these fields would lead to an appealing enhancement of the number of resolvable compounds.

Field-Flow Fractionation is in its nature such a cascading process¹, and in this review we will give a brief description of the theory behind the method as well as some examples of its experimental validity, involving the use of different types of fields.

II. THEORETICAL BACKGROUND

As the name Field-Flow Fractionation suggests, the method uses the coupling of a force-field with a flow profile to achieve differential migration (Figure 1). The carrier velocity, v , should be slow enough such that laminar flow is ensured, and the channel width is required to be small in order to give pronounced velocity differences with small differences in distance from the wall (i.e., $\frac{dv}{dx} \gg 0$ near the wall). The force-field, which can be electrical, magnetic, gravitational, thermal, or chemical, etc., in nature, causes the solute molecules to accumulate in a layer of unique thickness near one channel wall. The solute is then transported by flow along the channel at a rate fixed by the mean thickness of the layer.

The idea of such a generalized coupling between force-field and fluid-velocity profile was suggested by Giddings¹; it was proposed independently by Berg and Purcell¹⁰ for the special case of gravitational fields.

We now look at the origin, structure, and thickness of the solute layer, for this fixes the rate of solute migration. In the absence of flow, a force acting on a molecule will induce a certain average drift velocity, U , along axis x , which is linearly dependent on the field-strength and inversely so on the frictional resistance to motion. The flux of solute across a unit area normal to the field is influenced by the magnitude of U , the local sample concentration c , and the diffusion rate of the solute under study. Writing the diffusion coefficient as D , the flux J_x along the field direction x is described as follows

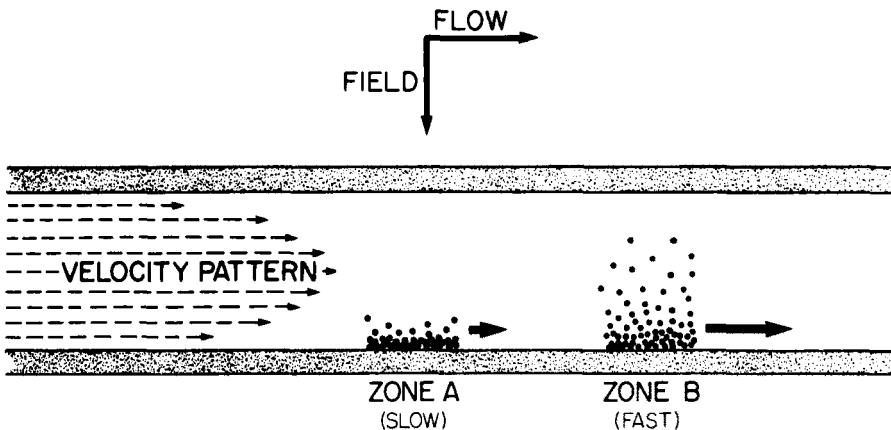


FIGURE 1

Illustration of the basic field-flow fractionation principle.

$$J_x = - D \frac{dc}{dx} + U \cdot c \quad (1)$$

which at steady state, where the net flux equals zero, leads to the expression

$$D \cdot \frac{dc}{dx} = U \cdot c \quad (2)$$

a differential equation with the solution

$$c = c_0 \exp \left(x \frac{U}{D} \right) \quad (3)$$

Since by our choice of coordinates, x measures the distance from the wall where solute accumulates, the drift velocity, U , is a negative quantity. Equation 3 can therefore be written as

$$c = c_0 \exp \left(-x \frac{|U|}{D} \right) \quad (4)$$

The constant c_0 is the concentration at the wall, $x = 0$, and $|U|$ is the absolute value of U .

Equation 4 can be rewritten as

$$c = c_0 \exp\left(-\frac{x}{\ell}\right) \quad (5)$$

where $\ell = \frac{D}{|U|}$ is a parameter with the dimensions of a length that is an effective measure of the thickness of the solute layer. Under most circumstances ℓ is equal to the average distance of solute molecules from the wall.

A given force-field will now cause molecules of different kinds (with different diffusion characteristics and different field susceptibilities) to distribute unequally in the field direction, x . For example, a large induced drift velocity, $|U|$, paired with a small diffusion coefficient, D , will lead to a small ℓ -value and hence a very compact wall-hugging zone. Conversely a small mobility together with a large D value will give rise to a large ℓ and a rather diffuse zone.

In FFF the unique lateral distribution of each solute, as measured by varying ℓ values, is coupled with the parabolic velocity profile of an axially flowing solvent. It stands to reason that molecules of a species with a thick layer having a large ℓ value will on the average follow faster stream lines than molecules of a species distributed in a thin layer characterized by a small ℓ . This coupling has been discussed in detail by Giddings² and we shall here touch on only parts of this discussion. The average zone velocity in the axial direction z is given by

$$v = \frac{\langle c(x) \cdot v(x) \rangle}{\langle c(x) \rangle} \quad (6)$$

$c(x)$ being the concentration distribution as expressed in equations 3, 4, and 5, and $v(x)$ the velocity profile of the

carrier liquid. For obvious reasons this profile has to be dependent on flow channel geometry. In the case of flow between two infinite parallel plates spaced a distance w apart, the flow profile takes the following analytical form¹¹

$$v(x) = \frac{\Delta P}{2\eta L} x \cdot (w-x) \quad (7)$$

where ΔP is the driving pressure, η the carrier viscosity and L the length of the channel. The average velocity, $\langle v \rangle$, is obtained through integration of equation 5 between the two channel walls, normalized by division with w

$$\langle v \rangle = \frac{\Delta P w^2}{12 \eta L} \quad (8)$$

The combination of equations 7 and 8 then gives us the working analytical expression for the velocity profile

$$v(x) = 6 \langle v \rangle \left(\frac{x}{w} - \frac{x^2}{w^2} \right) \quad (9)$$

The corresponding expression for the case of flow in a circular tube¹¹ is given by equation 10

$$v(x) = 2 \langle v \rangle \left[1 - \left(\frac{x}{R_o} \right)^2 \right] \quad (10)$$

in which R_o is the radius of the tube.

The mean carrier velocity, $\langle v \rangle$, can be measured experimentally by injecting into the flow stream a solute which is unaffected by the field. The measured retention time, t_r , of this "inert" solute yields $\langle v \rangle$ through the expression

$$\langle v \rangle = \frac{L}{t_r} \quad (11)$$

A quantity of great importance in differential migration methods involving flow is the relative migration rate or relative

retention parameter, R , of a solute zone. This quantity is defined as

$$R = \frac{V}{\langle v \rangle} ; \quad 0 \leq R \leq 1 \quad (12)$$

With the help of equations 12 and 6 we can now write an analytical express for R

$$R = \frac{\langle c(x) \cdot v(x) \rangle}{\langle c(x) \rangle \langle v \rangle} \quad (13)$$

When solved explicitly for flow between infinite parallel plates, R becomes⁴

$$R = (6\ell/w) [\coth(2\ell/w)^{-1} - 2(\ell/w)] \quad (14)$$

Figure 2 shows the variation of R with respect to the dimensionless quantity ℓ/w . It is seen that the smaller the ℓ value of a particular sample, the smaller is its retention parameter R and consequently the smaller its zone velocity. In the limit of very small (ℓ/w) 's, retention ratio R becomes linearly dependent on ℓ/w , implying that even relatively small deviations in ℓ give rise to considerable differences in migration rate.

$$\lim_{(\ell/w) \rightarrow 0} R = 6(\ell/w) \quad (15)$$

In common with other methods of separation, zone spreading in FFF is a process of great importance, affecting component resolution and overall peak capacity. Zone spreading can be characterized by the plate height, which is defined¹² as $H = \sigma^2/L$. The plate height can be described by the equation⁴

$$H = \frac{2D_1}{R\langle v \rangle} + \chi \frac{w^2\langle v \rangle}{D_2} + \sum H_i \quad (16)$$

FIELD FLOW FRACTIONATION

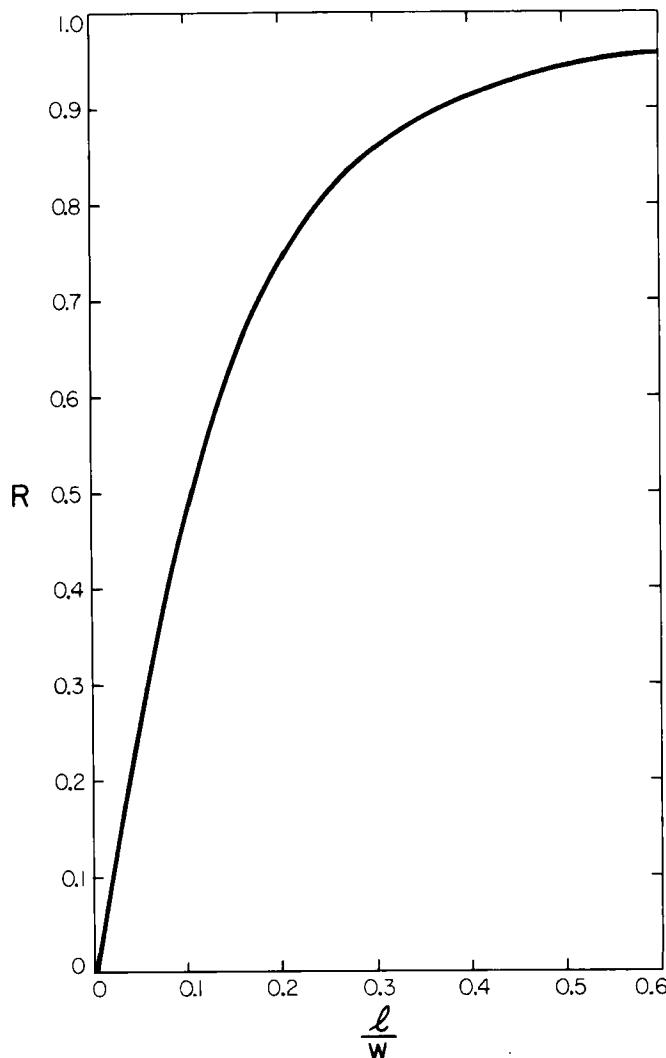


FIGURE 2

Variation of retention ratio R with l/w for an isothermal system.
(See Equation 14.)

In these expressions σ^2 is the variance of a solute zone just prior to elution and L , as above, the length of the field-exposed channel. The first of the three terms on the right hand side represents dispersion due to longitudinal diffusion, and is therefore dependent upon the time the zone spends in the channel, implying an inverse dependence on zone velocity $R(v)$. The second term reflects the departure from equilibrium due to the axial velocity, and is influenced by the geometry of the flow-channel. It is proportional to mean flow velocity $\langle v \rangle$, as in chromatography. The last term, finally, sums up the plate height contributions caused by other phenomena, such as the finite width of the injection slug, relaxation time (i.e., the time involved in the initial establishment of the lateral, field-induced, concentration profile), and zone dispersion occurring in the injector or detector.

The symbol D_1 in equation 16 represents the longitudinal diffusion coefficient. Ideally D_1 is constant across the channel. This condition is obviously not true in the case of solutes with important second virial coefficients, as are found in systems with large amounts of sample or with highly compressed zones (small ℓ 's). However the plate height concept is not truly applicable to these nonideal and therefore nonlinear systems. A linear case in which diffusivity is not constant occurs with thermal FFF, where the imposed temperature gradient causes a variation in the diffusive behaviour across the channel. In this case, D_1 is a cross-sectional average of local D values

$$D_1 = \frac{\langle D(x) \cdot c(x) \rangle}{\langle c(x) \rangle} \quad (17)$$

The parameter D_2 is an axial diffusion coefficient, which can be given the value D_1 or any other desired value with the proper choice of χ . In the case of TFFF it has been considered most tractable to use either the diffusion coefficient at the

zone's center of gravity (that is, at $x_{cg} = \langle x \cdot c(x) \rangle / \langle c(x) \rangle$), where concentration and temperature are easily obtained, or at the cold wall for even greater convenience. For most nonthermal methods of FFF, of course, $D_2 = D_1 = D(x)$.

The non-equilibrium coefficient, χ , has been discussed elsewhere^{2,4}. Some limiting cases are of major importance. In the limit of very thin solute layers between parallel plates

$$\chi = 24(\ell/w)^3 \quad (18)$$

$$(\ell/w) \rightarrow 0$$

In a laminar flow situation without field, χ varies from 1/96 for a channel with circular cross section, to 1/105 in the case of infinite parallel plates. Most of the experimental results have been obtained in channels with at least a 40:1 ratio between breadth and width. For practical purposes these can be approximated by the infinite parallel plate case. It is obvious from equation 16 that no matter what the geometry and ℓ/w value, the control of the axial solvent velocity is of utmost importance for the reduction of H and the resulting optimization of resolution and peak capacity.

Theory thus indicates that, under the influence of a force-field, different species in a flow channel will each form a zone with a characteristic "atmospheric height," ℓ , which will be coupled unequally with the velocity profile, thus providing the differential migration needed for resolution.

We will now proceed to discuss some applications of the above outlined theory.

III. ELECTRICAL FFF

The flow channel in this case has semi-permeable walls, perpendicular to the field direction, to allow the passage of

small ions while retaining macromolecules. The electrodes are well removed from the channel walls in order to insure a constant field across the channel without disturbing influences from the electrode reactions⁵. A macromolecule with mobility μ ($\text{cm}^2/\text{sec-Volt}$), exposed to a field of $E(\text{Volt/cm})$, will acquire a drift velocity

$$U = \mu E \quad (19)$$

Hence the "atmospheric height" of a zone of this particular solute in an electrical field-flow situation takes the form

$$l = \frac{D}{|\mu E|} \quad (20)$$

Since the mobility, and to some extent the diffusion coefficient, vary with pH, we can select conditions suitable for a particular separation problem (see Figure 3). This has, of course, always been a strong point of conventional electrophoretic techniques.

Advantages of EFFF include the fact that thermal effects are of minor consequence and applied voltages need not be excessively high.

Figure 4 shows the essential features of the EFFF method. One of the most severe experimental difficulties with this method is the acquisition of suitable membranes for the channel walls. Obvious requirements are that they be semi-permeable with a cut-off limit of around MW 10,000; that their permeability be non-selective on the ionic level; that they be uncharged; and finally that they be mechanically stable. Some regenerated cellulose membranes fulfill these requirements moderately well. When stretched, most membranes become flat--but obstacles in the flow channel, such as an occasional gas bubble blocking the outlet, tend to expand the channel volume and distort the parallel plate situation.

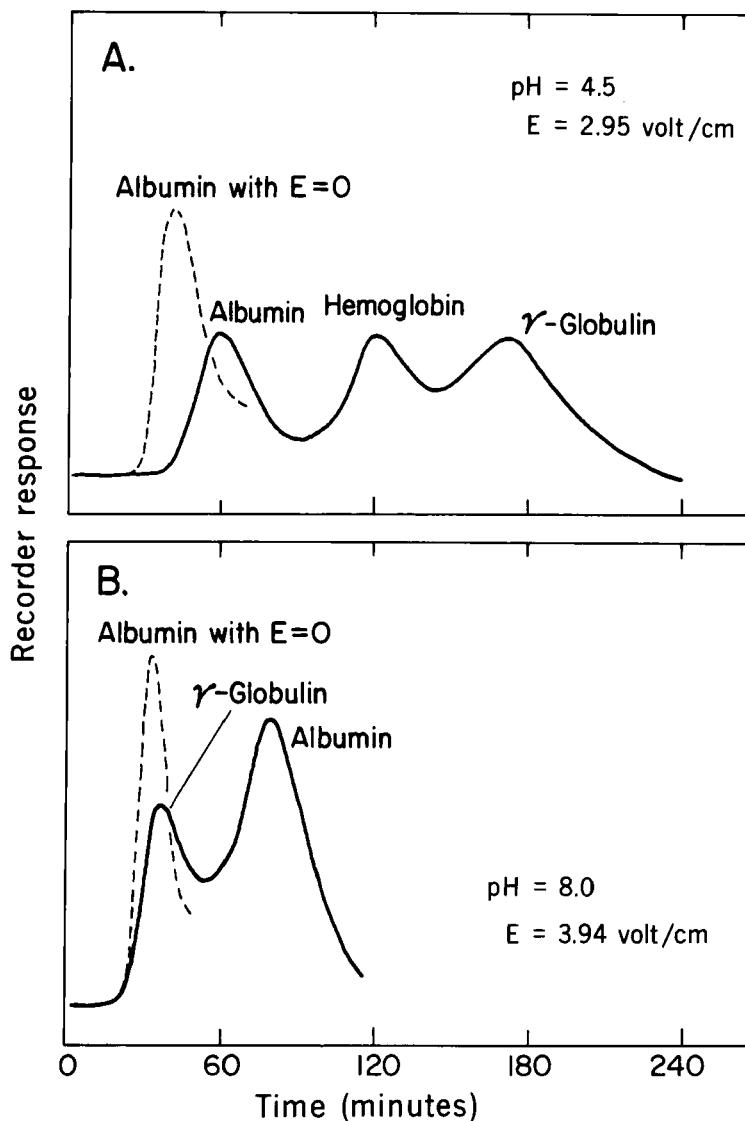


FIGURE 3

Protein separation by electrical field-flow fraction (EFFF). The order of elution of albumin and γ -globulin is reversed by changing from a $\text{pH} = 4.5$ in A to a $\text{pH} = 8.0$ in B.

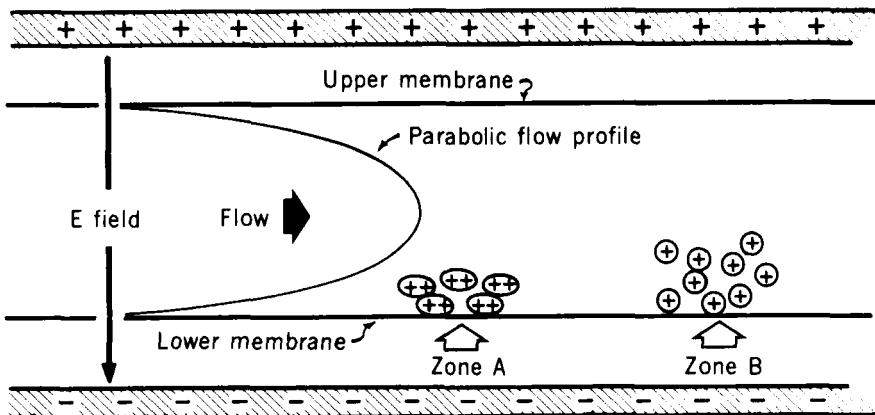


FIGURE 4

Diagrammatic representation of EFFF apparatus.

Figure 5 demonstrates that, in spite of existing experimental problems, our EFFF data are in reasonable agreement with theory. The parameter l/w always showed a linear relationship with $1/E$ as predicted; however in certain cases the lines cut the axes away from the origin, possibly due to selective permeation by the ionic constituents of the buffer solution.

IV. GRAVITATIONAL FFF

If we accommodate the flow channel inside a centrifugal basket, and spin it with an angular velocity, ω , the particles within this channel will experience a force, equal to

$$F(r) = (\rho - \rho_0) V \omega^2 r \quad (21)$$

where ρ is the density of the solute and ρ_0 that of the solvent; $\omega^2 r$ is the centrifugal acceleration at radius r ; and V is the

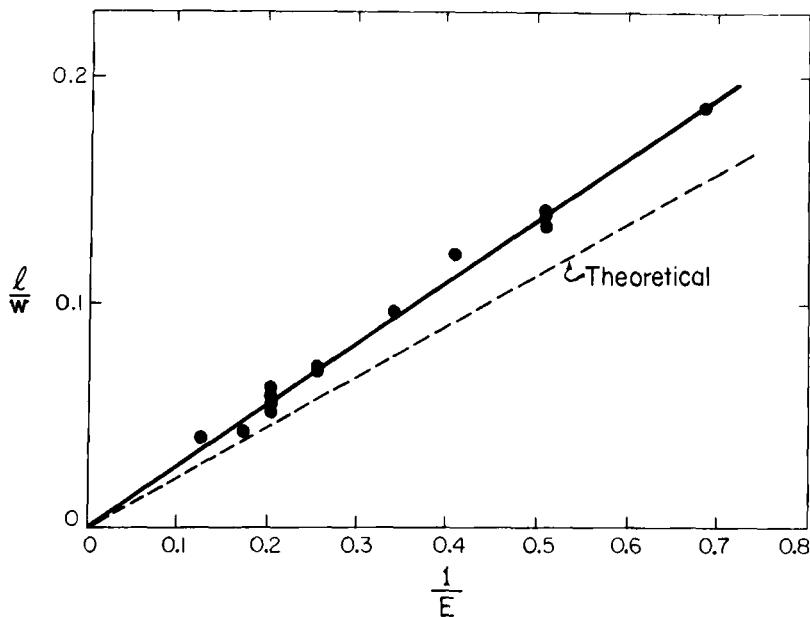


FIGURE 5

Illustration of electrical field-flow fractionation with albumin at pH = 8.0, plotting l/w versus $1/E$, where E is in volts per centimeter. Theoretical line calculated using $\mu = 7.3 \times 10^{-5} \text{ cm}^2/\text{sec-V}$ at 25° C .

molecular volume. Since the thickness of the channel is negligible in comparison with the rotor radius, $\omega^2 r$ is for all practical purposes constant across the channel and will be assigned the symbol G . If we express V in terms of molecular weight M and solute density ρ , the force equation takes the following form

$$F = \frac{M}{N} \cdot G \left(1 - \frac{\rho_0}{\rho}\right) \quad (22)$$

N being Avogadro's number. A particular solute with friction coefficient f will consequently assume a drift velocity of the following magnitude

$$U = \frac{F}{f} = \frac{M}{N f} \cdot G \left(1 - \frac{\rho_o}{\rho} \right) \quad (23)$$

The friction coefficient equals

$$f = \frac{k T}{D} \quad (24)$$

where k is Boltzmann's constant and T is the absolute temperature of the system. With this, the important parameter λ (equation 5) will have the following form

$$\lambda = \frac{D}{|U|} = \frac{D N k T}{|D G M (1 - \rho_o/\rho)|} = \frac{R T}{|G M (1 - \rho_o/\rho)|} \quad (25)$$

where R is the gas constant. There are obviously two solute specific parameters entering this equation: M and ρ . Three other parameters depend upon experimental conditions: T , G and ρ_o . The latter can be adjusted to optimize separation.

In no other experimental application of the Field-Flow technique does the channel geometry play as important a role as it does in GFFF. It has been observed^{14,15} that tubular flow inside a spinning duct gives rise to a secondary flow component, the magnitude of which depends upon the axial velocity. The pattern exhibited by secondary flow is illustrated in Figure 6. This diagram shows that the flow has a lateral direction; the fluid moves outward across the center of the tube and splits close to the outer wall into two peripheral back-fluxes. The net result of this effect is a symmetrical double spiraling motion forward in the axial direction. There is a narrow layer near the walls which, due to the viscosity of the liquid and its slow axial motion in the area, will not participate to any substantial degree in this helical movement. The larger the bulk velocity and the stronger the field, the narrower this zone will be, and the more pronounced will be the required lateral orientation of solute in the field in order to get any retention.

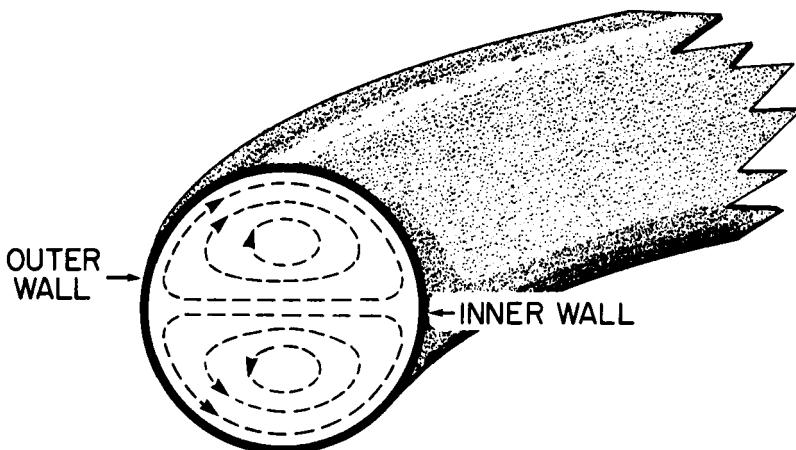


FIGURE 6

Secondary flow patterns in tubes of circular cross section.

With the help of Figure 6 we realize that a moderately large ℓ will lead to a most unfortunate zone spreading and a total departure from the basis of FFF theory. The "infinite parallel plate" geometry is far more helpful in this situation since the pressure difference generated between inner and outer wall is very small. In our case, the ratio of width to breadth is 1:40, which has proven to be quite adequate. With this type of channel, Mr. Frank Yang in this laboratory has achieved the differential migration of polystyrene beads ($\rho = 1.05$) of different sizes, using distilled water as the carrier¹⁶. A quite comforting correspondence with theory shows that the effects of secondary flow have become negligible (Figure 7). A series of experiments with virus particles (T2, MW 49×10^6 , $\rho = 1.57$ and T7, MW 240×10^6 , $\rho = 1.57$) in aqueous buffer solution also show good retention at very moderate angular velocities (2000 rpm).

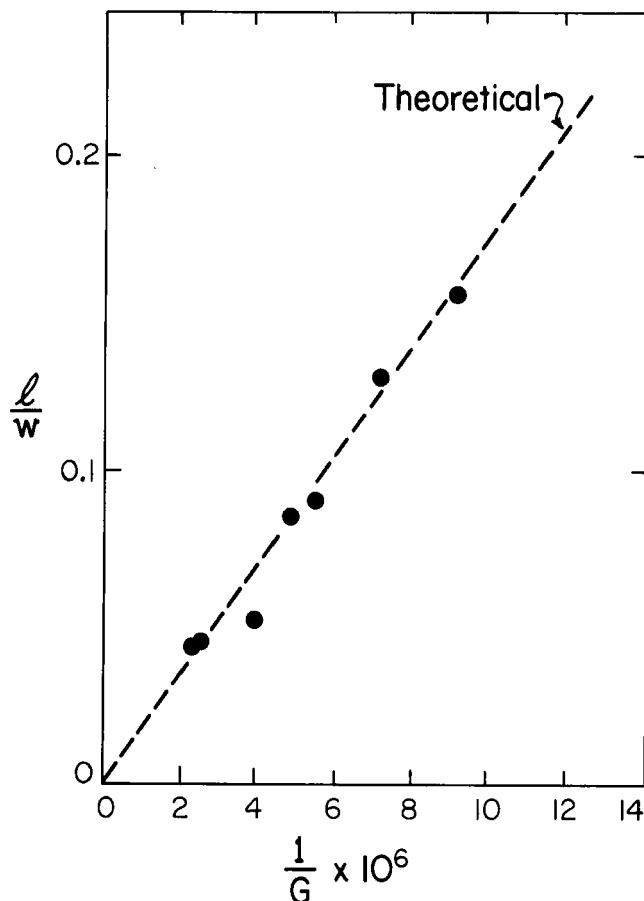


FIGURE 7

Parameters for gravitational field-flow fractionation (GFFF) using 0.109 μ polystyrene latex particles. G is expressed in cgs units.

In an independent series of experiments similar in scope to our GFFF system, Berg and Purcell succeeded in achieving retention of various particles in both the earth's gravitational field and in a laboratory centrifugal field^{17,18}. The former was realized in two ways: in a flat channel with laminar flow between two

glass plates and on one plate with an essentially static detergent film at the top of the flowing liquid layer. Separation occurred between monomers, dimers and trimers of latex beads 0.8μ and 1.3μ in diameter.

The centrifugal field was applied to a sample of bacteriophage RL7 (MW 4×10^6) contaminated with low molecular weight material. The rotor in this case was a long hollow cylinder revolving around a vertical axes. The liquid film was held in place by the field and its thickness was regulated by a barrier at the rotor bottom.

V. THERMAL FFF

From an experimental viewpoint, thermal FFF is the simplest and the most exploited of the field-flow methods^{3,4,19}. However, from a theoretical standpoint, it is the most complex member of the FFF family⁴. First of all, a temperature gradient across a channel ensures viscosity differences between top and bottom walls, leading to distortions in the parabolic velocity profile. Furthermore the ordinary Fickian diffusion coefficient is no longer constant across the channel, and in all likelihood the driving force of the system--the thermal diffusion--also varies. The solvent density also acquires a lateral gradation. For these reasons, the mathematical description of the concentration profile is not as straight forward as are those for previously discussed cases. Therefore for the sake of clarity we discuss the particular material flux equation which is pertinent to TFFF.

Introducing D' as the thermal diffusion coefficient, c_T as the total number of moles (solute plus solvent) per unit volume, and X as the solute mole fraction

$$J = - D c_T \frac{dX}{dx} - D' c_T X (1 - X) \frac{dT}{dx} \quad (26)$$

For dilute solutions $(1 - X)$ approximates unity. Since the temperature gradient implies a gradient in solution density, c_T will be variable with x . The solute concentration, c , equals $c_T X$, and its derivative with respect to x looks as follows

$$\frac{dc}{dx} = X \frac{dc_T}{dx} + c_T \frac{dx}{dx} \quad (27)$$

Substitution of this into Equation 26, and the substitution of c for $c_T X$ and α for $\frac{D'}{D} T$ leads to

$$J = - D \left[\left(\frac{dc}{dx} + c \frac{\alpha}{T} \frac{dT}{dx} \right) - \frac{c}{c_T} \frac{dc_T}{dx} \right] \quad (28)$$

After we transform the last term of equation 18 into a temperature sensitive form,

$$- \frac{c}{c_T} \cdot \frac{dc_T}{dx} = - \frac{c}{c_T} \cdot \frac{dc_T}{dT} \cdot \frac{dT}{dx} ,$$

we recognize the appearance of the coefficient for thermal expansion,

$$\gamma = - \frac{1}{c_T} \cdot \frac{dc_T}{dT}$$

which for practical purposes is negligible in TFFF, but which will be retained for the sake of generality in the solution of the flux equation. Under steady state conditions, where $J = 0$, Equation 28 can be rearranged to

$$\frac{d \ln c}{dx} = \left(\frac{\alpha}{T} + \gamma \right) \cdot \frac{dT}{dx} = \frac{1}{l} \quad (29)$$

which in its simpler and more practical form gives

$$l = \frac{T}{\alpha + \frac{dT}{dx}} \quad (30)$$

If we assume the temperature gradient to be linear, dT/dx can be replaced by $\Delta T/w$, which shows ℓ/w to be inversely dependent upon the total temperature difference, ΔT . In Figure 8 we present some observations on a polystyrene of 51,000 molecular weight in ethylbenzene. These polystyrene studies have been extended to solvents other than toluene with considerable success. However the nature of the solvent is quite important for the magnitude of the observed effect. Strong hydrogen-bonding systems, for

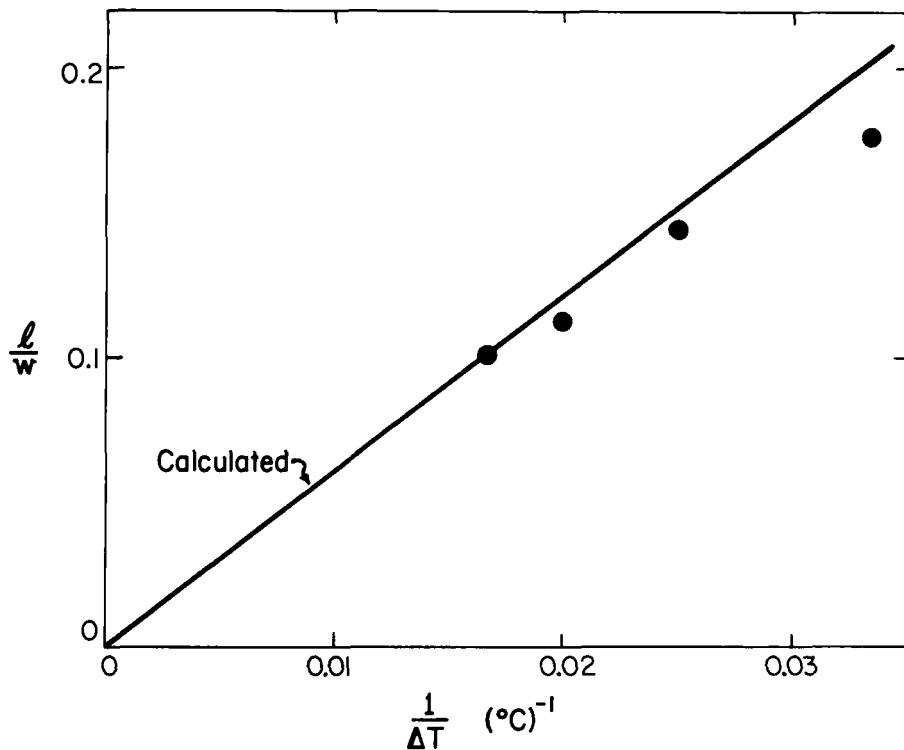


FIGURE 8

Retention characteristics of thermal field-flow fractionation with 51,000 molecular weight polystyrene in ethylbenzene.

instance, seem to provide only feeble thermal diffusion effects. Studies of a wide variety of proteins, nucleic acids and dextrans in our laboratory, all in aqueous buffer solutions, gave absolutely no retention. A Blue Dextran fraction of MW 2×10^6 was then selected for study in a series of aqueous mixtures of DMSO. No retention was observed until 60% DMSO, but as the DMSO concentration increased, the effect became more and more noticeable. Further studies are being pursued in which the water structure has been perturbed by various known structure breakers, such as guanidinium hydrochloride.

VI. PROGRAMMING TECHNIQUES IN FFF

In cases where the samples are very inhomogeneous with substantial differences in molecular weights, densities, electrophoretic mobilities, or other parameters affecting the drift velocities, it is necessary to apply a strong field at the beginning of a separation run to fractionate the least retained components. After the elution of a suitable number of column volumes, solutes which are only slightly or not at all affected by the field will have left the channel, and it becomes advantageous to reduce the field successively in order to speed the elution of highly retained components and thus to economize on time. A field reduction will in all cases lead to a steady increase in λ with a subsequent decrease in elution time (Figure 9). The force acting on a molecule can also be changed through a variation of some solute-related property such as electrophoretic mobility, reduced mass, or thermal diffusivity. Such variations will cause λ to go through a discontinuity (see Figure 10).

In case of EFFF, where (see Equation 20),

$$\lambda = \frac{D}{|\mu E|}$$

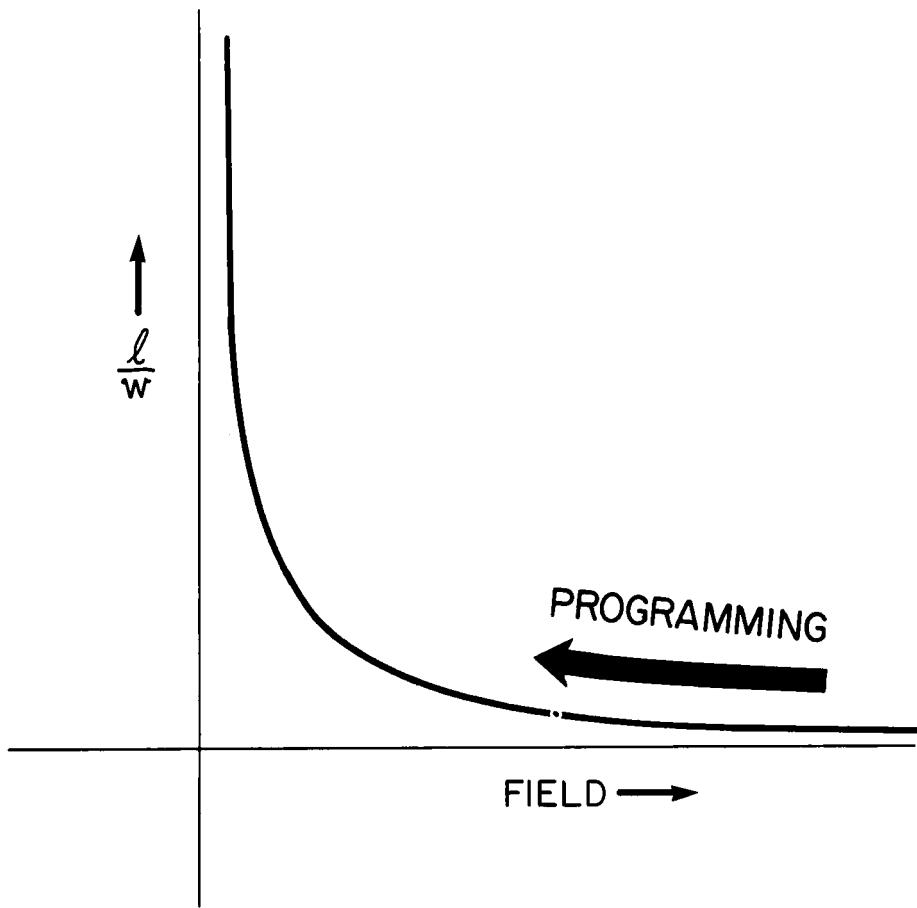


FIGURE 9

Variation of l/w with field strength, showing how programming with a decreasing field leads to a steadily increasing l/w .

we have several possibilities for variation of retention, as follows. First, a gradient in pH would, although not explicitly obvious from the equation, affect²⁰ D and thus l . More importantly, it would alter the mobility μ . Since the mobility of each solute goes through zero at the respective isoelectric point and then

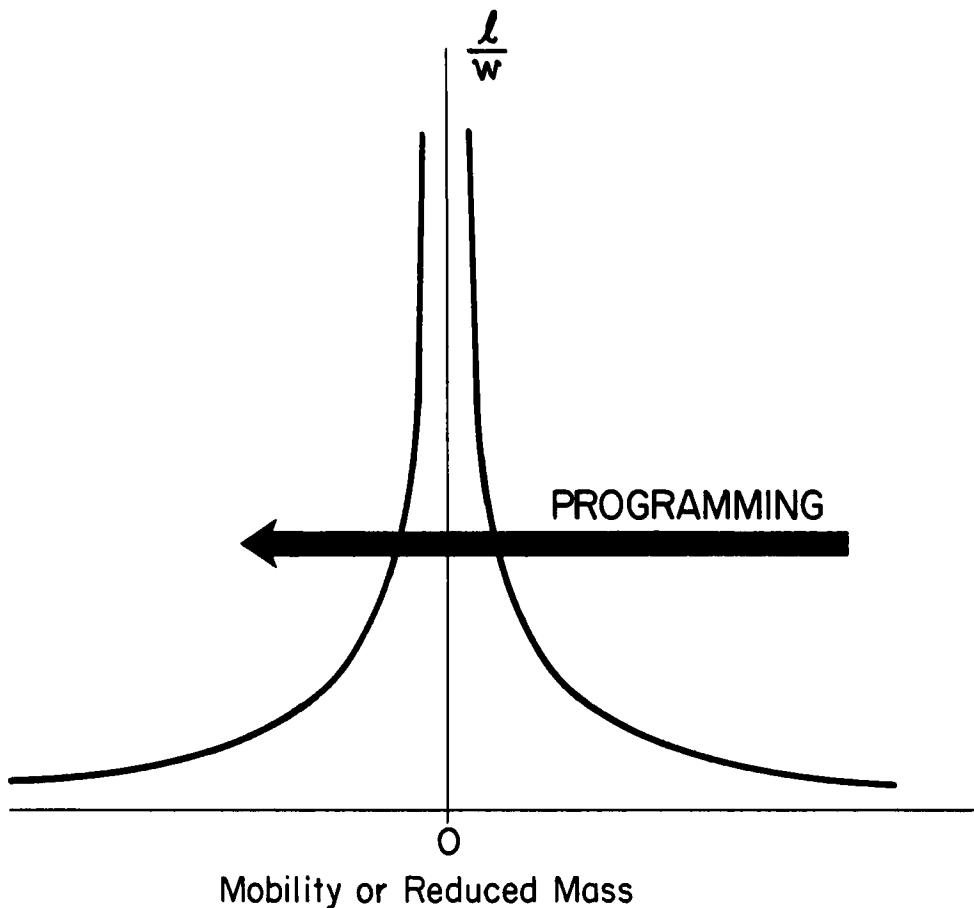


FIGURE 10

Variation of l/w with the solute related parameters, mobility and reduced mass, at constant field strength. Programming, if carried too far, will transfer solute to the opposite wall (at the place indicated by the discontinuity in the figure), and could lead to disruptive zone patterns.

increases again upon further change in pH, such a gradient would need to be restricted.

The temperature is another variable not explicitly entered in the expression for λ . The possible results of a temperature variation are, as in most FFF methods, of limited scope since the variability has to be confined to the liquid range of the solvent. Most appealing of all is the possibility of programming the electrical field strength, E , with temperature and pH kept constant.

The GFFF presents three parameters that can be altered by changing conditions, as is seen in equation 25, according to which

$$\lambda = \frac{T}{M(1 - \frac{p_0}{\rho}) \omega^2 r}$$

This equation shows that temperature, solvent density, or centrifugal acceleration are open to variation. As discussed above, obvious limits exist to the operable temperature range, which discourage a variation in T . On the contrary, both solvent density and centrifugal acceleration are quantities that easily lend themselves to variation. The case of a density gradient is similar in scope to that of a gradient in pH, but if carried too far would transfer solute to the opposite wall. More straightforward is a variation in $\omega^2 r$ or G , the centrifugal field.

As for the thermal field, where the thickness of the solute layer is expressed by

$$\lambda = \frac{T}{\alpha \frac{dT}{dx}} = \frac{T}{D' T \frac{dT}{dx}} = \frac{D}{D' \frac{dT}{dx}}$$

D and D' would be temperature dependent, but again a variation in average temperature, keeping the gradient dT/dx constant, has too limited an effect on λ . A programmed decrease in the

temperature gradient, however, has proven quite useful as a time saving operation³. It has been demonstrated experimentally that thermal diffusion differs with differences in solvent. It is therefore possible to imagine a solvent gradient in TFFF as a possible analog to the pH gradient mentioned above for EFFF.

VII. CONCLUSIONS

The Field-Flow Fractionation method shows considerable theoretical potential for macromolecular separations. Up to now the experimental hurdles in the path of achieving this potential have been only partially breached. However the method is so broad in scope that the failure of any one sub-technique for a given class of compounds leaves open the possibility of exploiting several others. The inherent advantages of the technique, combined with such a versatile variety of approaches, encourages optimism as to the ultimate role of FFF in macromolecular separations.

VIII. ACKNOWLEDGMENT

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