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Hyperlayer Field-Flow Fractionation

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Abstract

Hyperlayer field-flow fractionation is proposed as a method designed to overcome some of the limitations of conventional field-flow fractionation (FFF). In hyperlayer FFF, steady-state particle layers are formed above the channel wall by the combination of a primary field (e.g., sedimentation or electrical) and a secondary gradient (such as density of pH). Such zones could be separated along the flow axis in FFF even if they strongly overlap in the field or lateral direction. An approximate theory is derived for sedimentation hyperlayer FFF, showing both the rate of zone migration and the extent of peak broadening. Calculations are presented which show that the system should be highly effective for the separation of particles in the vicinity of 1 μm in diameter or larger.

INTRODUCTION

Field-flow fractionation is an elution separation technique utilizing flow through a narrow channel much like chromatography. Retention is induced by lateral fields or gradients rather than by a stationary phase. The method is most useful for the analytical-scale separation of macromolecules and small particles.

Field-flow fractionation (FFF), as commonly practiced, employs a lateral field or gradient to force sample particles against one wall of a flow channel (*1, 2*). The interplay of the applied force and of backdiffusion leads to an exponential layer whose approximate mean height above the wall is l, a parameter given by

$$l = D/U \tag{1}$$

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where D is the particle diffusion coefficient and U is the field-induced velocity. The ratio of l to channel thickness (or width) w is a dimensionless layer thickness termed λ:

$$\lambda = l/w = D/Uw \tag{2}$$

Quantity λ is the central parameter of FFF, determining both the magnitude of solute retention and the degree of zone broadening as measured by plate height.

Conventional FFF, as described above, has demonstrated considerable promise in separating, on the basis of differences in size, high molecular weight macromolecules and particles up to approximately 1 μm in diameter (*3, 4*). However, some complications are inevitable if one attempts to extend the methodology to particles even larger in size or to particles differing in density, while at the same time attempting to maintain or even enhance the conditions appropriate to high resolution. The principal difficulties are as follows.

1) With increasing size, the particle is prevented by its own finite diameter from reaching the wall. Normally, as the particle diameter increases, the particles are subject to increased interactions with the field and are forced more closely toward the wall, producing a smaller λ value and a higher retention. However, because of steric exclusion, the center of gravity of large particles cannot reach the wall; at some level of particle size an increasing diameter will be accompanied by an increasing mean distance from the wall. At the transition diameter, all selectivity is lost (*5*). At larger diameters, a reverse selectivity occurs in which the largest particles of a mixture are eluted first. This is termed steric FFF (*6*). While this inversion of retention order is often useful, it will occasionally work in opposition to the normal retention mechanism of FFF and thus prove counterproductive.

2) The smallest plate heights and thus the narrowest peaks are predicted to occur for the smallest possible λ values because these correspond to the thinnest solute layers. The value of λ can be made arbitrarily small by increasing the strength of the external field. However, this causes impractical increases in retention volume and retention time. It also tends to create a sample dilution problem which can make detection difficult for high retained peaks. Thus the beneficial effects of zone narrowing can only be achieved by concomitant sacrifices.

3) All walls have a microroughness that disturbs the flow profile in their vicinity. This leads to zone distortion and broadening. In addition, many particles tend to adhere to walls, particularly when they are forced into intimate contact by strong fields.

The above problems suggest that there would be considerable advantage to keeping the particle layer in an FFF channel away from the wall.

Several separation methods exist in which steady-state layers are formed without the aid of a wall. These methods have been termed equilibrium-gradient methods of separation (*7*). They include isoelectric focusing (*8*) and density gradient (or isopycnic) sedimentation (*9*). In these cases a strong primary field (electrical or centrifugal) impels each particle, but a secondary gradient (in pH or density) causes a reversal in the direction of the force acting on the particle at some equilibrium position (*7*). Therefore, particles of a given type tend to form equilibrium layers centered about the equilibrium point.

Other specific equilibrium-gradient methods have been suggested including those using strong thermal gradients and nonuniform electrical fields (*7*). These have not been developed as practical methods, however.

The principles employed in equilibrium-gradient separations can be used to form steady-state layers above the wall in FFF channels. For each of the four equilibrium gradient approaches mentioned above, corresponding FFF systems can be constructed. In this report we focus only on the application of this concept to sedimention FFF (which gives us sedimentation hyperlayer FFF), but the principles and theory would be much the same for the other suggested methodologies.

Experimental implementation of sedimentation hyperlayer FFF requires careful and extensive instrument modification relative to normal sedimentation FFF. The concepts and technique have been under development in our laboratory since an initial disclosure in 1977 (*10*); a special apparatus has been constructed and is now undergoing preliminary testing. A method equivalent to sedimentation hyperlayer FFF was recently described by a former colleague (*11*).

The principles by which steady-state layers are formed in sedimentation FFF are illustrated in Fig. 1. These layers can be termed hyperlayers to distinguish them from the layers commonly formed against the lower channel wall. Species i, for example, will form a hyperlayer centered about coordinate position $x_{eq,i}$ at which the particle density of i is matched by the density of the background fluid. Species j, having particles of a different density, will form layers centered about another coordinate position $x_{eq,j}$. We note that these layers need not be fully separated from one another in hyperlayer FFF, although such separation is necessary in equilibrium gradient methods of separation. A large degree of overlap can be tolerated between the hyperlayers of FFF because the zones displace differentially along the channel (flow) axis and will eventually separate along this new coordinate as long as a modest increment exists in their respective mean velocities. Thus hyperlayer FFF promises to enhance resolution well above the values possible with ordinary equilibrium-gradient methods.

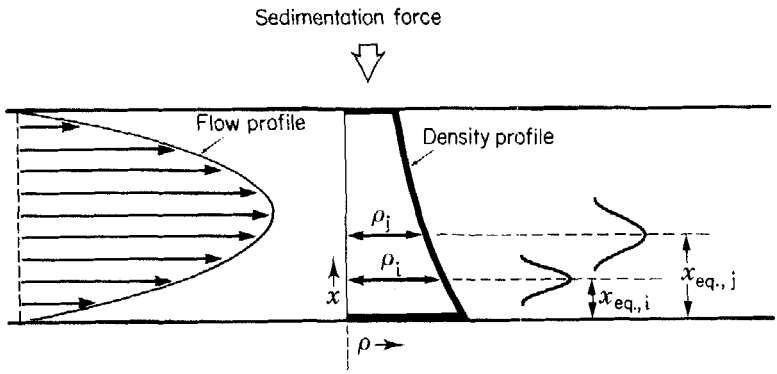

FIG. 1. Principles of sedimentation hyperlayer FFF, a method in which steady-state layers are formed away from the wall. Species i and j, with densities ρ_i and ρ_j, seek equilibrium levels $x_{eq,i}$ and $x_{eq,j}$, where the density of the channel fluid matches their own. They form narrow layers centered around those levels. The concentration across a layer peaks at the equilibrium level then decreases rapidly toward either side as indicated by the bell-shaped curves.

The background density gradient can in theory be formed from many different types of dispersed materials added to the carrier. However, because of the relatively weak fields usually employed in sedimentation FFF, and because of the small channel thickness w, relatively large species are required to form a high gradient. Simple electrolyte or sucrose solutions, using low molecular weight species, are unsuitable. Ideal, perhaps, are the small silica particles marketed under the name Ludox by DuPont. These particles have diameters of about 100 Å or more. This material has been employed successfully for cell and other separations using density-gradient sedimentation. We calculate that for the dimensions typically used in sedimentation FFF, an effective λ value of unity (for the density-forming particles, not for the sample) will be formed with particles of approximately 130 Å. A gradient corresponding to $\lambda = 0.5$–1 should be close to optimum for such systems.

ZONE FORMATION

We present here a very approximate theory of zone migration rate and zone width in hyperlayer FFF. This theory will give some indication of the feasibility of the method. A more rigorous formulation will follow.

Zone Migration

The mean downstream displacement velocity $\mathscr{V}$ of a given species will be approximated very closely by the fluid flow velocity v at the equilibrium elevation x_{eq} for that species: $\mathscr{V} = v(x_{eq})$. Thus, in a channel formed by two infinite parallel walls a distance w apart, the zone velocity of a species will be given by

$$\mathscr{V} = 6\langle v\rangle \left[\left(\frac{x_{eq}}{w}\right) - \left(\frac{x_{eq}}{w}\right)^2 \right] = 6\langle v\rangle[\Gamma - \Gamma^2] \qquad (3)$$

where $\langle v\rangle$ is the mean fluid velocity and Γ is the dimensionless elevation x_{eq}/w. This equation is based on the assumption of a parabolic flow profile and this, in turn, is predicated on a constant viscosity. Variations in viscosity can be treated by a method (or related methods) developed in this laboratory for thermal FFF in order to obtain a more accurate expression for zone velocity. This will be dealt with in a subsequent paper. Retention ration R—the ratio of zone velocity $\mathscr{V}$ to mean fluid velocity $\langle v\rangle$—is obtained directly from Eq. (3):

$$R = \frac{\mathscr{V}}{\langle v\rangle} = 6[\Gamma - \Gamma^2] \qquad (4)$$

It is seen from this equation that R values can exceed unity by a factor as large as 1.5 for solutes at the midpoint of the channel, $\Gamma = 0.5$. The maximum R value in conventional FFF is unity.

Zone Broadening

We now turn to the subject of zone width. First we note the implication of Fig. 1 that narrow bell-shaped profiles describe the concentration across the thickness of solute hyperlayers. It can be shown that these profiles are Gaussian and that the variance along coordinate x is given by the equation (*7, 12*)

$$\sigma_x^2 = kT/VG(d\rho/dx) \qquad (5)$$

where k is Boltzmann's constant, T is temperature, V is the volume of the sample particle, G is acceleration, and $d\rho/dx$ is the density gradient of the fluid along the lateral axis.

As sample particles are carried down the channel in their respective hyperlayers, individual particles will make irregular Brownian excursions across the thickness of the layer. The relaxation time for such excursions determines the dispersion of solute along the flow axis z. An analogous situation applies in conventional FFF where Brownian excursions occur over the exponential layer next to the wall. For the latter case the limiting equation for plate height can be written

$$H = 4l^2 \mathscr{V} / D \tag{6}$$

where D is the particle diffusion coefficient. In a very approximate way, we can employ a random walk model for hyperlayer dispersion similar to that employed for dispersion in exponential layers, from which the form of Eq. (6) can be obtained (*1*). We assume that diffusion carries solute particles back and forth over distance $\pm m\sigma_x$, where m is a parameter of order 2. The mean time necessary for such diffusional excursions is approximately

$$t_D = (2m\sigma_x)^2/2D = 2m^2\sigma_x^2/D \tag{7}$$

The length of a random step is the distance gained (or lost) by particles traveling at one of the extremes $\pm m\sigma_x$ for time t_D:

$$\mathscr{L} = (dv/dx)(m\sigma_x)t_D \tag{8}$$

where dv/dx is the gradient in flow velocity along axis x, and must be assigned the value it acquires at zone equilibrium position x_{eq}. The number n of random steps taken is simply $t(\text{elution})/t_D$, but since the elution time equals column length L over mean solute velocity $\mathscr{V}$, n becomes

$$n = L/\mathscr{V} t_D \tag{9}$$

According to the random walk model, the variance along flow axis z, σ_z^2, is $\mathscr{L}^2 n$. With the help of the three previous equations, this becomes

$$\sigma_z^2 = 2m^4\sigma_x^4 L(dv/dx)^2/\mathscr{V} D \tag{10}$$

In that plate height H in a uniform channel can be defined by σ_z^2/L, we have

$$H = 2m^4\sigma_x^4(dv/dx)^2/\mathscr{V} D \tag{11}$$

For the parabolic flow profile assumed here and used in Eq. (3), velocity gradient dv/dx at elevation x is

$$\frac{dv}{dx} = \frac{6\langle v\rangle}{w}\left(1 - \frac{2x}{w}\right) \tag{12}$$

The value of this gradient at $x = x_{eq}$ or, equivalently, at dimensionless altitude $\Gamma = x_{eq}/w$, is simply

$$(dv/dx) = 6\langle v\rangle(1 - 2\Gamma)/w \tag{13}$$

When this and Eq. (3) are substituted into Eq. (11), H becomes

$$H = \frac{12m^4\sigma_x^4\langle v\rangle}{w^2D}\,\frac{(1 - 2\Gamma)^2}{\Gamma(1 - \Gamma)} \tag{14}$$

With the substitution of Eq. (5), this acquires the form

$$H = \frac{12m^4\langle v\rangle}{w^2D}\left(\frac{kT}{VGd\rho/dx}\right)^2\frac{(1 - 2\Gamma)^2}{\Gamma(1 - \Gamma)} \tag{15}$$

If D is written as kT/f', where f' is the friction coefficient per molecule, Eq. (15) becomes

$$H = \frac{12m^4\langle v\rangle kTf'}{(wVGd\rho/dx)^2}\,\frac{(1 - 2\Gamma)^2}{\Gamma(1 - \Gamma)} \tag{16}$$

If the solute particles are spheres of diameter d, Stokes' law, $f' = 3\pi\eta d$, can be used for f', where η is the viscosity at x_{eq}. The particle volume V is $\pi d^3/6$. With these substituted into Eq. (12), H is

$$H = \frac{1296m^4}{\pi}\,\frac{\eta kT\langle v\rangle}{(wGd\rho/dx)^2d^5}\,\frac{(1 - 2\Gamma)^2}{\Gamma(1 - \Gamma)} \tag{17}$$

which is our final equation. The flow profile part of this expression (the Γ-containing term) is, of course, approximate because of the assumption of constant viscosity.

DISCUSSION

We note that the plate height H of Eq. (17) is proportional to flow velocity $\langle v \rangle$ in a way that is characteristic of conventional FFF and of chromatography. It is also proportional to viscosity which reflects the commonly observed inverse dependence of H on diffusivity.

The most notable feature of Eq. (17) is the proportionality of H to the inverse fifth power of particle diameter. According to this dependence, larger and therefore more sluggish particles will generate a considerably smaller H than will smaller particles. The reason for this is simply that larger particles tend to form more compact layers such that the Brownian excursions are shorter and can be accomplished more rapidly. In addition, the velocity increment across a compact zone is very small so that there is little tendency for dispersion in the first place.

Furthermore, Eqs. (15)–(17) show that H can be reduced rapidly by increasing acceleration G and the density gradient $(d\rho/dx)$.

In order to estimate the order of magnitude of plate heights that can be expected, we assume the following specific values for the system: $m = 2$, $d = 10^{-4}$ cm, $\langle v \rangle = 10^{-2}$ cm/s, $\eta = 10^{-2}$ P, $G = 10^{6}$ cm/s^2, $(d\rho/dx) = 10$ g/cm^4, $w = 0.025$ cm, $\Gamma = 0.25$, and $T = 300$ K. These values inserted into Eq. (17) yield an H value of 0.6×10^{-4} cm. Such a system would thus yield over 10^6 plates per meter, and considerably more for large particles. While it is anticipated that the experimental realization of such high plate numbers would be exceedingly difficult or impossible due to other system limitations, the above calculation shows the enormous potential in generating theoretical plates in hyperlayer FFF methods. The high plate counts would, of course, be accompanied by exceptional resolution and peak capacity. Therefore, by using this approach, it should be possible to separate cells and other particles from one another based upon rather minimal differences in their densities.

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REFERENCES

1. J. C. Giddings, *J. Chem. Educ., 50,* 667 (1973).
2. J. C. Giddings, *Anal. Chem., 53,* 1170A (1981).
3. J. C. Giddings, M. N. Myers, K. D. Caldwell, and S. R. Fisher, in *Methods of Biochemical Analysis*, Vol. 26 (D. Glick, ed.), Wiley, New York, 1980, p. 79.
4. J. C. Giddings, S. R. Fisher, and M. N. Myers, *Am. Lab.*, p. 15 (May 1978).
5. J. C. Giddings, *Sep. Sci. Technol., 13,* 241 (1978).

6. J. C. Giddings and M. N. Myers, *Ibid., 13*, 637 (1978).
7. J. C. Giddings and K. Dahlgren, *Sep. Sci., 6*, 345 (1971).
8. H. Svensson, *Acta Chem. Scand., 15*, 325 (1961).
9. M. Meselson, F. W. Stahl, and J. Vinograd, *Proc. Natl. Acad. Sci. U.S., 43*, 581 (1957).
10. University of Utah Patent Office file no. U-728: *Hyperlayer Field-Flow Fractionation*, by J. C. Giddings.
11. J. Janca, *Makromol. Chem., Rapid Commun., 3*, 887 (1982).
12. J. C. Giddings, in *Treatise of Analytical Chemistry*, Part I (I. M. Kolthoff and P. J. Elving, eds.), Wiley, New York, 1981, Section G, Chap. 3, p. 63.