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(1973) 'Parameters for Optimum Separations in Field-Flow Fractionation', Separation Science and Technology, 8: 5, 567 — 575

To link to this Article: DOI: 10.1080/00372367308057046

URL: http://dx.doi.org/10.1080/00372367308057046

SEPARATION SCIENCE, 8(5), pp. 567–575, 1973

Parameters for Optimum Separations in Field-Flow Fractionation

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Abstract

Parameters that yield optimum separations in field-flow fractionation (FFF) are investigated. Expressions for minimum plate height and optimum velocity are derived. It is shown that a typical FFF column is theoretically capable of yielding 12,000 plates per foot. With increasing retention, plate height decreases and optimum velocity increases. Minimum time conditions, analyzed next, are related to the rate of generation of theoretical plates. The latter increases with the rate of molecular transport and, surprisingly, with retention. Practical hurdles to achieving an infinite rate of generation of plates by going to infinite retention are discussed. Finally, a comparison is made between optimum separations using FFF and using direct fields (electrophoresis, sedimentation, and related methods.)

INTRODUCTION

In an earlier paper a physical basis was established for retention and plate height in field-flow fractionation (*1*). In particular, attention was focused on the important limiting case in which zones were considerably retained. The condition for this limiting case is expressed by $R \ll 1$, where R is the retention ratio—the ratio of zone velocity $\mathscr{V}$ to mean carrier flow velocity $\langle v \rangle$. If one assumes that the velocity profile is parabolic within a flattened channel of width w (a channel between two flat plates that are theoretically of infinite extent), then in the limiting retention case some very simple equations arise for retention and plate height (*1–3*):

$$R = 6\lambda \tag{1}$$

$$H = \frac{2D}{\mathscr{V}} + \frac{4l^2\mathscr{V}}{D} + \sum H_i \tag{2}$$

where D is the diffusion coefficient, l is the mean height of the solute layer in a zone, λ equals l/w, and $\sum H_i$ is the sum of various plate height terms arising in external dead volumes, relaxation processes, and solute heterogeneity.

In this paper we shall explore the implications that these and related equations have in achieving separation. In particular we shall examine the magnitude of the minimum achievable plate height, the optimum velocity, the potential speed of separation, and the relative efficiency of FFF and other methods of separation.

MINIMUM PLATE HEIGHT AND OPTIMUM VELOCITY

In a FFF column that is functioning with a minimum of extraneous disturbances, the plate height terms represented by $\sum H_i$ may be neglected. In this case the plate height is

$$H = \frac{2D}{\mathscr{V}} + \frac{4l^2\mathscr{V}}{D} = \frac{B}{\mathscr{V}} + C\mathscr{V} \tag{3}$$

This is similar in form to the expression for capillary columns in chromatography, but the velocity term differs in one significant detail. Velocity here is the mean zone velocity, $\mathscr{V} = R\langle v \rangle$, whereas in chromatography the mean solvent velocity, $\langle v \rangle$, is universally used. Nonetheless coefficient B is identical in the two cases because the respective velocities are reflective of the length of time spent in the mobile phase (the only phase in FFF) where the bulk of diffusion occurs.

The minimum plate height (with respect to velocity changes) achievable in FFF is obtained by setting the derivative, $dH/d\mathscr{V}$, equal to zero. This minimum, and the corresponding optimum velocity, are given by

$$H_{\text{min}} = 2\sqrt{BC} = \sqrt{32}l = 5.66l \tag{4}$$

$$\mathscr{V}_{\text{opt}} = \sqrt{B/C} = D/\sqrt{2}l = 0.707D/l \tag{5}$$

Since l is not directly observable, it is desirable to seek alternate expressions. One approach is to use the limiting retention expression of Eq. (1): $R = 6\lambda = 6(l/w)$. This yields

$$l = Rw/6 \tag{6}$$

When this is substituted above, $H_{\min}$ becomes

$$H_{\min} = \frac{\sqrt{32}}{6} Rw = 0.94Rw \cong Rw \tag{7}$$

In words, the minimum plate height is now approximately equal to the product of two observable parameters: the retention ratio R and the column width w. This expression shows that $H_{\min}$ drops rapidly with increasing retention (decreasing R). Hence one expects to get the best resolution for the most highly retained peaks. These are ordinarily the high molecular weight component of the mixture.

In a typical case we may expect a zone with a retention of $R = 0.1$ to be migrating in a column of width $w = 0.025$ cm. The minimum plate height, from Eq. (7), would be $H_{\min} = 0.0024$ cm or 24 μ. This would provide over 12,000 plates/foot, a very satisfactory efficiency indeed.

Substitutions also provide insight into variations of the optimum velocity, $\mathscr{V}_{\text{opt}}$. When the limiting expression from Eq. (6), $l = Rw/6$, is substituted into Eq. (5), we get

$$\mathscr{V}_{\text{opt}} = \sqrt{18}\frac{D}{Rw} \tag{8}$$

Since $\mathscr{V}_{\text{opt}} = R\langle v\rangle_{\text{opt}}$, the optimum mean velocity of the carrier fluid is

$$\langle v\rangle_{\text{opt}} = \sqrt{18}\frac{D}{R^2 w} \tag{9}$$

This equation shows $\langle v\rangle_{\text{opt}}$ to be greatest for columns of narrow width, w. It shows also a significant trend with changes in retention parameter R. Highly retained components exhibit relatively large $\langle v\rangle_{\text{opt}}$ values because of the inverse square dependence on R. The dependence on diffusion coefficient D may ameliorate this effect slightly, because in many systems low R values go hand in hand with high molecular weight and thus low D values. However, at the most, D only partially offsets the significant relationship between $\langle v\rangle_{\text{opt}}$ and R suggested by Eq. (9).

The foregoing results suggest that a flow programming system might be useful for FFF. The early peaks could be eluted under low-flow conditions and for successive peaks the flow rate could be increased. This would not only keep the system closer to optimum resolution throughout the run, but it would also speed up the highly retained peaks which might otherwise require an inordinate time for elution.

Another way to express $\mathscr{V}_{\text{opt}}$ is attained by using the basic formula (*2*) for l

$$l = D/U \tag{10}$$

where U is the drift velocity—the mean molecular velocity induced by the field. When this is substituted for l in Eq. (5), we obtain the equations

$$\mathscr{V}_{\text{opt}} = U/\sqrt{2} \tag{11}$$

$$\langle v \rangle_{\text{opt}} = U/\sqrt{2}R \tag{12}$$

The topmost expression shows that the optimum rate of travel of the peak is just slightly below the field-induced drift velocity.

Some numbers are useful at this point. A typical macromolecule under conditions of moderately high retention may be associated with parameters in the vicinity of $D = 10^{-7}$ cm^2/sec, $w = 0.025$ cm, and $R = 0.1$. Various equations above can be used to obtain other approximate parameters: $l = 0.0004$ cm $(4\ \mu)$, $U = 0.00024$ cm/sec, and $H_{\text{min}} = 0.0024$ cm. The optimum zone velocity, Eq. (11), is therefore approximately 0.0002 cm/sec or 0.7 cm/hr. This velocity is very low. It suggests that the principal challenge in developing FFF methodology is to obtain reasonable separation speed. It must be kept in mind that macromolecular separations are inherently slow in any system because of their sluggish transport characteristics. Hence the need to speed up macromolecular separation processes is generally crucial. Next we explore this topic for FFF.

SEPARATION SPEED IN FFF

If retention conditions are specified (preferably corresponding to favorable conditions), any given separation requires some minimum number of theoretical plates, N_{min}, for its successful realization. Equations are available for specifying the number of plates needed to obtain either some minimum resolution between two peaks, (*4*, *5*) or some minimum number of peaks resolvable in a single run (the peak capacity) (*5*, *6*). The time required to meet these minimum conditions is the time needed to generate the N_{min} theoretical plates. This time is

$$t = N_{\text{min}}/\dot{N} \tag{13}$$

where $\dot{N}$ is the rate of generation of plates. Minimum time, of course, is achieved only with a maximal level of $\dot{N}$.

To an approximation, a theoretical plate is generated every time molecules in different streamlines have an opportunity to intermix (*7*). The time required for this, as pointed out in the previous paper (*1*), is the time needed to diffuse a distance $2l$

$$t_p \sim \frac{(2l)^2}{2D} = \frac{2l^2}{D} \tag{14}$$

By this simple model the number of plates that can be generated per unit of time is simply the reciprocal of the time, t_p, needed to generate a single plate

$$\dot{N} = \frac{1}{t_p} \sim \frac{D}{2l^2} \tag{15}$$

Thus separation speed increases with increasing diffusivity and with increasing compression of the solute layer.

The full conditions needed to maximize $\dot{N}$ are best seen by proceeding more rigorously. The method is parallel to that used for chromatography (*4*). Quantity $\dot{N}$ can be written as

$$\dot{N} = \frac{\mathscr{V}}{H} = \frac{R\langle v\rangle}{H} \tag{16}$$

Use of the plate height expression in Eq. (3) leads to

$$\dot{N} = \frac{1}{(B/\mathscr{V}^2) + C} \tag{17}$$

With the help of Eq. (5) this reduces to

$$\dot{N} = \frac{1/C}{(\mathscr{V}_{\text{opt}}/\mathscr{V})^2 + 1} \tag{18}$$

When the peak velocity equals the optimum velocity, $\mathscr{V} = \mathscr{V}_{\text{opt}}$, $\dot{N}$ equals $1/2C$. However as $\mathscr{V}$ exceeds $\mathscr{V}_{\text{opt}}$, $\dot{N}$ approaches its maximum value

$$\dot{N}_{\text{max}} = 1/C = D/4l^2 \tag{19}$$

where the latter form was obtained using the definition of C from Eq. (3). This equation has the same functional form as the more simply-derived Eq. (15). Both equations suggest the desirability of maximizing D and minimizing l. Derivation of the latter equation shows, in addition, the desirability of operating above the "optimum" flow velocity.

Some alternate forms of Eq. (19) are instructive. If D/U is used in place of l (Eq. 10), we get

$$\dot{N}_{\text{max}} = U^2/4D \tag{20}$$

This equation shows the importance of maximizing the field-induced drift velocity, U. It also appears to contradict Eq. (19) in suggesting a minimum diffusivity D, but in fact it does not do so. Both D and U are inversely related to the friction coefficient of molecules, f. Changes in solvent are reflected in parameter f, and this in turn affects D and U. Equations (19) and (20) both agree that

$$\dot{N}_{\max} = \text{constant}/f \tag{21}$$

so that in either case a minimum f (maximum D as well as U) is desired. This is best attained by using a low molecular-weight, nonviscous carrier at the highest practical temperature.

Another useful form of $\dot{N}_{\max}$ is obtained by substituting $l = Rw/6$ from Eq. (6) into Eq. (19). This yields

$$\dot{N}_{\max} = 9D/R^2w^2 \tag{22}$$

This shows, in addition to the above, that the potential rate of generation of plates increases dramatically with decreases in both retention ratio R and column width w. The inverse dependence on w^2 is fully expected. Any reduction in the dimensions over which diffusion must occur will speed the diffusive interchange of solute. The same is true, of course, in chromatography (*4*).

Unlike chromatography, FFF is theoretically capable, according to Eq. (22), of generating plates faster with highly retained peaks than with moderately retained peaks. One might expect the opposite result; slower-moving peaks will generate fewer plates per second, all other things being constant. But all other factors are not constant in FFF: highly retained solute peaks are compressed by the field into very thin layers. The reduced diffusion distance (and therefore time) required to traverse this layer explains the dramatic increase in the rate of acquiring plates.

In theory, $\dot{N}_{\max}$ could be pushed to infinity by increasing retention (decreasing R) and decreasing w. Several practical limits eventually intercede to prevent this ultimate achievement—an infinitely fast separation of arbitrary difficulty. Attempts to reduce R are limited, first of all, by the finite strength of the external field. Second, an infinitely compressed solute layer requires an infinitely smooth surface over which to glide. A significant disturbance of the system will occur when surface hills and gullies (or the size of the molecules themselves) exceed l in size. Third, a highly compressed solute layer implies either a very high solute concentration, with attendant nonlinearities, or vanishingly small samples. Fourth, the pressure needed to maintain flow above $\mathscr{V}_{\text{opt}}$ will ultimately increase to impractical levels.

Attempts to reduce column dimensions are similarly limited; increased field strengths, smoother surfaces, smaller samples, and greater pressure drops are necessary to maintain the same retention ratio R in the face of a decreasing w. It will be interesting to see which of these limitations eventually poses the most significant barrier to further increases in $\dot{N}_{\text{max}}$.

Equation (22) can be used to estimate a typical (not ultimate) value to be expected for $\dot{N}_{\text{max}}$. Assuming, as in the previous examples, that $D = 10^{-7}$ cm^2/sec, $w = 0.025$ cm, and $R = 0.1$, then Eq. (22) yields a value for $\dot{N}_{\text{max}}$ of 0.14 plates/sec, or about 500 plates/hr. For smaller macromolecules, $D = 10^{-6}$ cm^2/sec, this would increase to 5000 plates/hr. These values, if obtainable in practice, are highly acceptable for separation in inherently sluggish systems of macromolecules.

FFF AND DIRECT-FIELD METHODS

We have made occasional reference above to the comparative characteristics of FFF and chromatography. Here we shall undertake the comparison of FFF with direct-field (axial-field) methods of separation. A direct-field method is one, like electrophoresis or centrifugation, that causes differential migration in the field direction. In FFF the field and the axis of separation are perpendicular to one another. These methods, despite essential differences, can be compared on the basis of theoretical plate generation.

In a direct-field method, drift velocity U becomes a simple measure of the rate of progress of zones in the field direction. Plate height is simply obtained by the definition $H = \sigma^2/L = 2Dt/Ut$, or (*5*)

$$H_{\text{min}} = 2D/U \qquad \text{(direct field)} \tag{23}$$

This assumes that no convective currents or other adverse influences are at work. For comparison the optimized plate height of FFF can be obtained by combining Eq. (4) and (10), giving

$$H_{\text{min}} = \sqrt{32}D/U \qquad \text{(FFF)} \tag{24}$$

The ratio of the two is

$$\frac{H_{\text{min}}(\text{FFF})}{H_{\text{min}}(\text{DF})} = \sqrt{8} = 2.8 \tag{25}$$

This shows that under equivalent conditions of diffusivity and field strength, the direct-field (DF) methods have a minimum plate height that is 2.8 times lower than that of FFF.

As pointed out earlier, the maximum rate of generation of theoretical plates, $\dot{N}_{\max}$, is a more significant criterion than $H_{\min}$ for separation efficacy in macromolecular systems. For direct-field methods $\dot{N}_{\max}$ is simply U/H, or, with the aid of Eq. (23)

$$\dot{N}_{\max} = U^2/2D \qquad \text{(DF)} \tag{26}$$

Equation (20) gives the comparable expression for FFF

$$\dot{N}_{\max} = U^2/4D \qquad \text{(FFF)} \tag{27}$$

Consequently the ratio is

$$\frac{\dot{N}_{\max}(\text{FFF})}{\dot{N}_{\max}(\text{DF})} = \frac{1}{2} \tag{28}$$

showing that direct-field methods are capable of producing plates twice as rapidly as FFF systems under equal conditions of field strength and diffusivity.

The foregoing results suggest that direct-field systems have a slight edge over the FFF approach. However, they do not reflect some serious handicaps of certain direct-field methods, nor do they reflect several advantageous characteristics of FFF.

From an experimental point of view, FFF is an elution technique, having inherent advantages in sample collection and analysis. More important from a fundamental point of view, FFF permits the more efficient use of available applied fields and in many cases allows the utilization of higher maximum field strengths (and thus U's). Increasing field strength, as shown by the role of U in Eq. (24) and (27), very rapidly makes up for the slight inherent disadvantages of FFF.

Some examples of the advantageous utilization of fields by FFF can be simply illustrated.

1. The maximum field strength in centrifugation occurs only at the outmost bounds of the rotating system; much of the separation takes place at lesser field closer to the axis. In gravitational FFF one can coil the flow tube flat against the outer perimeter, thus using the maximum field strength at all times.

2. The electrical field strength applicable to electrophoresis is limited by thermal effects. However, thermal effects in electrical FFF are not disadvantageous (*8*); indeed one method of FFF (thermal) relies on the

existence of a strong temperature gradient and its associated thermal diffusion.

3. FFF is effectively a multistage process, in the sense that the same field is used over and over again as components migrate along the channel. Reuse of the field makes it possible to achieve separation with much smaller potential drops (*9*). A potential drop of merely 1 V over a 0.25-mm wide FFF column will provide the same field intensity (and hence comparable results) as 2000 V used on a 50-cm electrophoresis strip. The FFF column can then be extended to any desired length, without additional potential drop (of course an increased current is required), to achieve any desired number of theoretical plates (*9*). This feature is particularly advantageous with thermal diffusion. It can be shown that direct thermal diffusive separations are virtually impossible because the largest practical temperature drops (around 100°C) are incapable of providing adequate fractionation. By contrast, thermal FFF is capable of producing multicomponent polymer separations (*10*).

The foregoing analysis shows that FFF has a substantial theoretical potential. The crucial question, which we have only started to explore, is whether (and when) this theoretical efficacy can be fully converted into practical utility.

Acknowledgment

This investigation was supported by Public Health Service Research Grant GM 10851–16 from the National Institutes of Health.

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Received by editor February 24, 1973