

CHROM. 10,745

## FINE-PARTICLE SEPARATION AND CHARACTERIZATION BY FIELD-FLOW FRACTIONATION

J. CALVIN GIDDINGS, MARCUS N. MYERS and JOHN F. MOELLMER

*Department of Chemistry, University of Utah, Salt Lake City, Utah 84112 (U.S.A.)*

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### SUMMARY

Field-flow fractionation (FFF) is described as a one-phase form of chromatography and its advantages are summarized. Its applicability to colloids and fine particles is considered. A brief discussion of FFF principles and theory is focused on retention and plate height, pointing to the unique fact that in FFF increased retention leads to a considerable decrease in plate height. Two sub-techniques, sedimentation FFF and flow FFF, are discussed at length because they have shown the greatest promise for particle separation and characterization. Examples of the former are presented, showing that mass and diameter information can be extracted directly from the fractogram. Attention is also given to the consistency of results within the sedimentation FFF system. It is shown that variations in field strength and flow-rate can be used to control retention and resolution. These controls demonstrate the flexibility of the system with respect to analysis time and resolving power. In a brief discussion of flow FFF the remarkable mass range capability of this sub-technique is noted.

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### INTRODUCTION

Field-flow fractionation (FFF) is an analytical-scale separation technique that is capable of fractionating species with molecular weights up to  $10^{12}$  or more<sup>1</sup>. It is a method dynamically similar to chromatography, but separation occurs in one phase instead of two, and it has therefore been called one-phase chromatography<sup>2</sup>. Unlike separations in most chromatographic systems, FFF separation is more readily achieved as the molecular weight increases, so that FFF is complementary to chromatography.

FFF gives effective fractionations in the molecular weight range  $10^3$ – $10^6$ , and is exceptional in the range  $10^6$ – $10^{12}$ . The latter is roughly the mass range of colloidal particles, and it includes most systems of fine particles. The fractionation and analysis of such particles is an important aspect of modern technology and environmental control<sup>3–6</sup>.

The advantages of FFF have been discussed previously<sup>1,7,8</sup>. They include the minimal surface area and reduced surface interactions that stem from the one-phase nature of the system; the precise theoretical relationship between retention and size parameters; the flexible, external control of retention; versatile programming; a large

mass or molecular weight range obtainable in a single column; and a high potential resolving power in the macromolecular-particulate range. The method is extremely versatile in its ability to deal with ionic and non-ionic solutes, aqueous and non-aqueous solvents, and a broad molecular weight range. Preliminary applications include organic polymers<sup>9,10</sup>, water-soluble polymers<sup>11</sup> and proteins<sup>7</sup>, in addition to the particulate systems that are the main subject of this paper.

For the purposes of our discussion, and in accord with accepted definitions, a fine particle is a particle with a diameter (aerodynamic) of  $3\ \mu\text{m}$  or smaller<sup>4</sup>. However, the meaning of the term *particle* is elusive in clearly defining our subject area. Webster's Third New International Dictionary notes that a particle (disregarding the elementary particles of nuclear physics) is "a very small portion of something material; minute quantity; tiny fragment". This is very broad and is not substantially narrowed by most other dictionary definitions. Cadle<sup>6</sup> asserts that "the term 'particle' can be defined as any object having precise physical boundaries in all directions...". If these boundaries are relatively constant in time, this would imply that particles are essentially rigid, thus excluding, for example, flexible chain polymers. We shall maintain this exclusion as a point of departure for the work of this paper. We shall also, for practical purposes, exclude single molecular species such as proteins.

Given a collection of particles as defined above, FFF is a system that will sort them according to mass or size, *i.e.*, a pulse of particles injected into its flow stream will emerge as a spectrum graded according to particle mass or size. The grading takes place according to precise mathematical laws. The graded spectrum can be stretched out or shortened, according to uniform or non-uniform programs, in order to enhance the resolution in selected regions. This is done entirely by the manipulation of external controls.

The principal objectives of this paper are (1) to demonstrate the existence of fine-particle fractionation using FFF, (2) to show the general agreement between FFF theory and experiment, (3) to show that particle mass and size can be extracted from FFF data and (4) to illustrate how the resolution and analysis speed are influenced by changes in flow-rate and field strength.

## BASIS OF FFF SEPARATIONS

### *Principles*

FFF is similar to chromatography in operation and in basic theory, but it differs in that it operates strictly in one phase in an unobstructed, thin, rectangular channel. As a solute-solvent mixture moves down the channel, an external gradient or force field, applied perpendicularly to the channel axis, interacts with the solute, forcing it into a characteristic equilibrium distribution layer against one wall. This layer is of different thickness for each distinct chemical or particulate species, depending on the physical basis of the coupling between the field and species and on the diffusion coefficient peculiar to that species.

Flow along the axis of the channel displaces the solute particles downstream. Because the flow is parabolic, its velocity is greatest near the center of the channel. Thus solutes with a thick layer extending into the center will be swept out of the channel first, while species forced into a narrow layer in the relatively quiescent flow regions near the channel wall will be significantly retained. This is the basis for

selective retention, and produces in general an elution spectrum in which small particles are eluted first and large particles last<sup>8,12-14</sup>.

The field responsible for the formation of solute layers (and thus for retention) can be of any form in which there is an interaction with the solute to produce a lateral motion of the solute species. Several sub-techniques of the basic FFF concept have been developed, each predicated on a different external field. Sedimentation FFF is based on centrifugal or gravitational force<sup>15-18</sup>, whereas electrical FFF and thermal FFF rely on electrical potential differences<sup>19-21</sup> and a temperature differential<sup>9,22-24</sup>, respectively. The effective lateral field in flow FFF is another solvent stream passing at right-angles to the axial flow through the semi-permeable upper and lower walls of the channel<sup>7,12,25-27</sup>.

The sub-techniques of FFF differ from each other in their range and general scope of applicability owing to the different natures of the interactions between solutes and specific fields. Taken together, however, these methods promise outstanding generality. In principle, FFF should be applicable to any colloidal material for which a disperse phase or solvent can be found.

The most fruitful sub-techniques for particle analysis are sedimentation FFF and flow FFF. We shall discuss these methods in more detail after presenting some of the quantitative considerations and equations that are needed to characterize FFF systems.

### *Theory of retention*

The concentration,  $c$ , of molecules in the equilibrium solute layer decreases exponentially above the accumulative channel wall according to the expression<sup>8,13,14,28,29</sup>

$$c/c_0 = \exp(-x/l) \quad (1)$$

where  $c_0$  is the concentration at the wall,  $x$  is the distance above the wall and  $l$  is a characteristic parameter with the dimension of length that is called the mean layer thickness. For convenience, we use the ratio of  $l$  to the column width,  $w$ , to express a dimensionless mean layer thickness,  $\lambda$ :

$$\lambda = l/w \quad (2)$$

The retention ratio,  $R$ , defined as in chromatography as the ratio of peak velocity to void peak velocity, is a function only of  $\lambda$ :

$$R = 6\lambda [\cot^2(1/2\lambda) - 2\lambda] \quad (3)$$

Under conditions of high retention, this equation assumes the simple form

$$R = 6\lambda \quad (4)$$

These relationships have been verified experimentally in many of our studies<sup>15,27</sup>. The parameter  $\lambda$  is related to the underlying physical chemistry of the system through the relationship

$$\lambda = D/Uw = kT/F_1 w \quad (5)$$

where  $D$  is the diffusion coefficient of the solute,  $U$  is the mean lateral drift velocity induced by the gradient or field,  $k$  is Boltzmann's constant,  $T$  is absolute temperature and  $F_1$  is the effective force exerted on a single particle by the field. Exploitation of eqns. 4 and 5 leads to information on the diffusivity, Stokes diameter and mass of the separated particles. We shall return to this later.

The retention volume,  $V_r$ , of an FFF peak is given by  $V_r = V^0/R$ , where  $V^0$  is the void volume. With the aid of eqn. 3, this becomes

$$V_r = V^0/6\lambda [\coth(1/2\lambda) - 2\lambda] \quad (6)$$

It can be shown that this equation reduces to the following expression for highly retained components<sup>11</sup>:

$$\frac{V_r}{V^0} = \frac{1}{6\lambda} + \frac{1}{3} = \frac{F_1 w}{6 k T} + \frac{1}{3} \quad (7)$$

where the latter form derives from eqn. 5. This equation is valid to within about 6%, even at two void volumes ( $V_r = 2V^0$ ), and the error rapidly decreases thereafter<sup>25</sup>. Thus  $V_r$  becomes linear in the force,  $F_1$ , of interaction of the field with the particle. At high  $V_r$  values the additive term,  $1/3$ , can be neglected, and  $V_r$  is then essentially proportional to  $F_1$ . The simplicity of these results facilitates the interpretation of FFF data.

We note that particles of relatively large size (*ca.* 1  $\mu\text{m}$ ) will be retained slightly less than described by the theory above because their centers of gravity cannot approach closer than one particle radius to the wall. In this instance  $R$  is described by the approximate relationship<sup>30</sup>

$$R = 6\alpha + 6\lambda \quad (8)$$

where  $\alpha$  is the ratio of particle radius to channel width,  $w$ .

#### Plate height

Plate height theory is moderately well developed but is complex. To a good approximation, the plate height,  $H$ , can be expressed in a form analogous to the equation governing chromatography in capillary tubes<sup>31</sup>:

$$H = \frac{2D}{R\langle v \rangle} + \frac{\chi w^2 \langle v \rangle}{D} + \Sigma H_t = \frac{B}{\langle v \rangle} + C \langle v \rangle + \Sigma H_t \quad (9)$$

where the three terms on the right-hand side represent longitudinal molecular diffusion, non-equilibrium processes and FFF non-idealities including end-effects, respectively. The parameter  $\langle v \rangle$  is the mean carrier velocity and  $\chi$  is a very complex function of  $\lambda$  (ref. 32). The terms  $B$  and  $C$  are used to show the similarity of the equation to those of chromatography.

Eqn. 9 is valid after an initial relaxation period in which the equilibrium distribution of particles is approached, provided that conditions can be maintained near equilibrium during the runs<sup>32</sup>. The same condition applies to chromatographic

theory<sup>31</sup>, which is analogous to FFF theory. Krishnamurthy and Subramanian have developed a more comprehensive theoretical approach<sup>33</sup> and other workers have extended the theory to allow for finite particle size<sup>34,30</sup>.

The middle term on the right-hand side of eqn. 9, the non-equilibrium or mass transfer term, imposes the ultimate theoretical limitations on column speed and efficiency<sup>13</sup>. We note that the coefficient,  $\chi$ , of this term assumes the following limiting form under conditions of substantial field strength and therefore substantial particle retention<sup>35</sup>:

$$\chi(\lambda) = 24\lambda^3 = R^3/9 \quad (10)$$

Using eqns. 10 and 5, we find that the plate height contribution of the non-equilibrium term is

$$H = (24D^2/U^3 w) \langle v \rangle \quad (11)$$

or

$$C = 24D^2/U^3 w \quad (12)$$

One important property of eqns. 10–12 is that they produce a considerable decrease in plate height with increasing retention. This contrasts with chromatography, where the plate height changes only slightly with variations in retention.

The reason why the plate height is expected to decrease with increasing retention is that the highly retained components have solute layers that are strongly compressed against the channel wall. The thinness of the layer facilitates rapid equilibration (mass transfer). The magnitude of the effect is substantial, as can be seen from the third power dependence in eqn. 10. A doubling of the retention (equivalent to a halving of  $R$ ) leads to an 8-fold decrease in  $\chi$ . There is some offsetting effect, up to a first-power dependence on  $R$  at most, due to changes in the diffusion coefficient,  $D$ . Even with this factor, the downward trend in plate height with increasing retention is considerable.

The decrease in plate height with increasing retention has been recognized as one of the advantages of FFF separations<sup>13</sup>. Nonetheless, experimental results testing this conclusion have been mixed. It is our belief that imperfections in the channel uniformity and surface often create departures from theory, especially at high retention. We shall present some recent results bearing on this question later in this paper.

In the next two sections we focus on the two FFF sub-techniques that have shown the greatest promise for particle fractionation and characterization: sedimentation FFF and flow FFF.

## SEDIMENTATION FFF

A centrifugal force was first proposed as one of several fields that could control retention in FFF systems<sup>36</sup>. Our experimental approach to this concept has been simply to wrap an FFF channel in a circular configuration within a centrifuge basket, extended out against its wall. Special seals have been designed to conduct solvent in and out of the spinning system. Additional details are available elsewhere<sup>15</sup>.

The theory of sedimentation FFF has been developed as an offshoot of general FFF theory<sup>15,16</sup>. It has been extended to include programming methods in which the field strength is changed continuously during the run<sup>15,18</sup>. With or without programming, the basic equation for  $\lambda$  is

$$\lambda = \frac{k T}{G m (1 - \rho/\rho_s) w} \quad (13)$$

where  $m$  is the particle mass,  $\rho_s$  is its density,  $\rho$  is the solvent density and  $G$  is the field strength in units of acceleration. This equation can be substituted into either eqn. 6 or 7 in order to relate retention volume,  $V_r$ , to particle mass,  $m$ . In the latter instance we obtain

$$\frac{V_r}{V^0} = \frac{G w (1 - \rho/\rho_s) m}{6 k T} + \frac{1}{3} \quad (14)$$

This equation, like its parent, eqn. 7, is valid only as a limiting form at high retention, but for practical purposes it can be employed for  $V_r \geq 2V^0$ .

For spherical particles, for which the mass,  $m$ , is related to the diameter,  $d$ , by  $m = \pi d^3 \rho_s/6$ , the retention volume can be related to  $d$  in place of  $m$ :

$$\frac{V_r}{V^0} = \frac{\pi}{36} \cdot \frac{G w (\rho_s - \rho) d^3}{k T} + \frac{1}{3} \quad (15)$$

This, too, is an approximate form valid for  $V_r \geq 2V^0$ .

Eqns. 14 and 15 show that particle mass,  $m$  (and for spheres the particle diameter,  $d$ ), can be calculated directly from the retention volume,  $V_r$ , and various physical parameters of the system. Especially important is the fact that  $V_r$  is a linear function of  $m$ . Consequently, an elution curve becomes a mass distribution curve without further manipulation of data, and one can therefore think of the elution curve beyond two void volumes as a linear mass spectrum of the particulate species. (One must be careful, however, to use a detector whose response is proportional to the mass, or if desired to the number, of particles in the detector cell, irrespective of particle size.)

The foregoing relationships have been tested with polystyrene latex beads and found to be reasonably accurate<sup>15,16,18</sup>. Here we shall describe some applications, including examples of separations, to establish a better understanding of the capability of the sedimentation sub-technique of FFF.

In the simplest case, one may wish to determine the mass or molecular weight of particles in a monodisperse particulate system. In virus systems, for example, it is often important to know molecular weights accurately. Most methods are tedious, indirect and require expensive equipment. Earlier we showed that sedimentation FFF can be applied conveniently to this problem<sup>17</sup>. Fig. 1 shows the results obtained in measuring the molecular weight,  $M$ , of T2 virus at different field strengths ( $\lambda$ ) and sample concentrations. The relative constancy of  $M$  with  $\lambda$  demonstrates the self-consistency of the method. This type of "internal" consistency test is always possible in FFF using variations in field strength.

Polydisperse particulate systems are more challenging. In order to obtain an

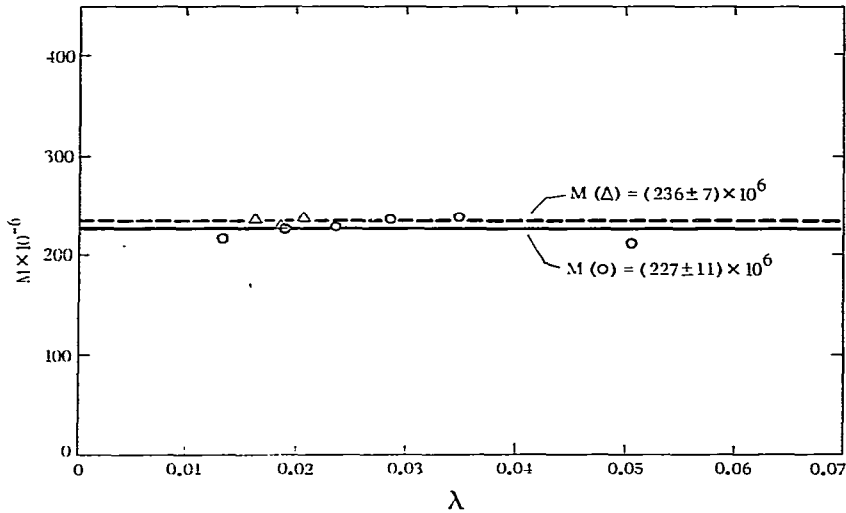


Fig. 1. Experimental molecular weights for T2 virus derived from sedimentation FFF data acquired at various  $\lambda$  values and with sample sizes of 0.2 mg (O) and 0.1 mg ( $\Delta$ ).

accurate mass distribution curve, band spreading within the column must be minimal. The separation of distinct monodisperse peaks also requires a narrow band width. Thus, if one can obtain good resolution for different monodisperse samples, it is safe to assume that accurate mass distributions can be obtained for polydisperse materials. We concentrated on the former because in working with monodisperse systems we have the additional advantage of being able to characterize instrumental performance.

Fig. 2 illustrates the ability of sedimentation FFF to separate monodisperse samples of polystyrene latex beads over a 10-fold size range and a 1000-fold mass range<sup>18</sup>. We are aware of no other results of comparable resolution. The high resolution indicates that the system would yield a valid mass distribution curve within the range of sizes indicated. With changes in field strength, the range could be extended in either direction. The fractogram in Fig. 2 was obtained with the aid of programming; without programming, a reduced size range would have resulted.

#### Consistency of FFF results

We now report recent results acquired from the same sedimentation FFF system as that which produced Fig. 2. The channel was 0.90 m in length, 0.25 mm in width and had a void volume,  $V^0$ , of 5.21 ml. The experimental system has been described more fully elsewhere<sup>15,18</sup>. The particles used were three polystyrene latex solids from Polysciences Inc., with reported diameters of 0.358  $\mu\text{m}$  with a standard deviation of 0.0068  $\mu\text{m}$ , 0.275  $\mu\text{m}$  with a standard deviation of 0.003  $\mu\text{m}$  and 0.183  $\mu\text{m}$  with no reported standard deviation. In addition, we used as a standard a sample of polystyrene latex beads of reported diameter 0.357  $\mu\text{m}$  from Dow Chem.

Our procedure was to inject the sample and then to halt the flow for a given stop-flow time while relaxation to equilibrium occurred. Flow was then resumed until the last peak was fully eluted.

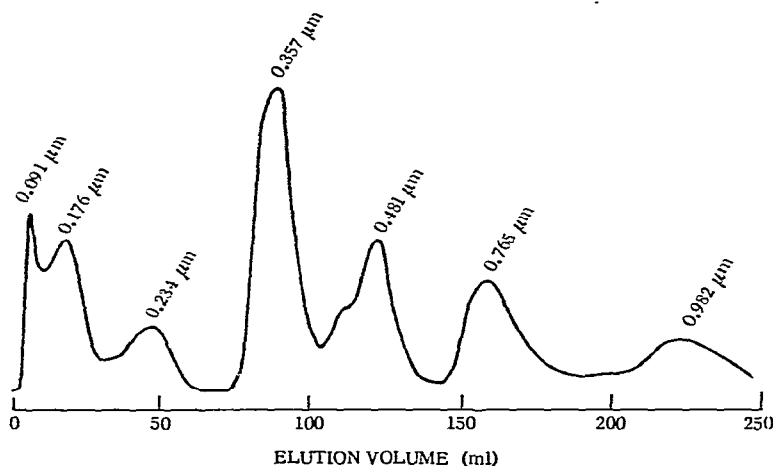


Fig. 2. Separation of polystyrene latex beads by sedimentation FFF. The beads have a 10-fold diameter range and a 1000-fold mass range.  $\dot{V} = 14.5$  ml/h. Initial rpm, 1714; final rpm, 365.

A useful check on the self-consistency of the apparatus can be made by means of a plot of  $\lambda$  versus  $1/G$  (ref. 15). Eqn. 13 shows that this plot should yield a straight line through the origin with a slope determined by the particle mass,  $m$ . The quantity  $\lambda$  can be obtained from observed retention values using our basic retention expressions (eqns. 3, 6, 7 or 8).

Fig. 3 shows the  $\lambda$  versus  $1/G$  plots thus obtained. The  $\lambda$  points for the nominal 0.358- $\mu\text{m}$  Polyscience beads and the nominal 0.357- $\mu\text{m}$  Dow beads are corrected

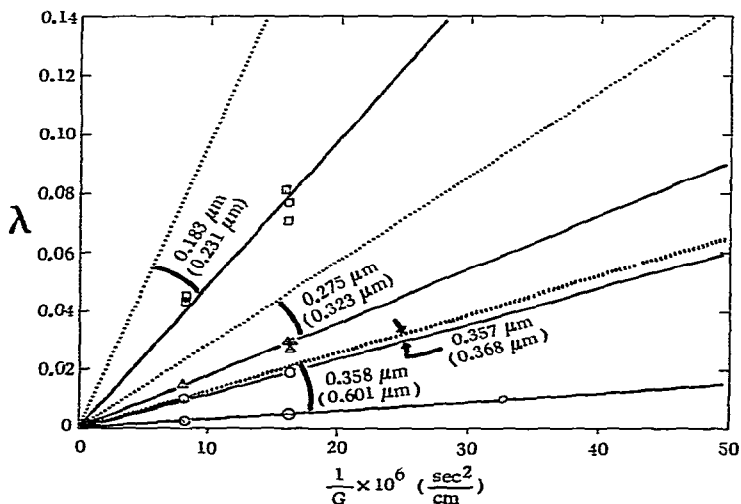


Fig. 3. Plots of  $\lambda$  versus the reciprocal field strength,  $1/G$ . The solid lines are empirical, fitted to the data. Each is associated with a dotted line calculated on the basis of the particle size reported by the supplier. The supplier's particle diameter is given as the upper number of each pair and the diameter determined by the sedimentation FFF data is the lower number, given in parentheses. Close agreement exists only for the Dow 0.357- $\mu\text{m}$  particles. All Polyscience particles show a significant discrepancy, as discussed in the text.



values, allowing for finite size effects using eqn. 8. The experimental points for all bead sizes fall on lines plotted through the origin, in accord with theory.

However, when we calculated the slopes of the empirical lines for the Polyscience beads in Fig. 3, they were inconsistent with the theoretical slopes calculated from eqn. 13. The theoretical slopes are shown as dotted lines in Fig. 3. We therefore attempted to make size measurements using an electron microscope (Cambridge Scientific Model Mark II-A). Table I shows a comparison of particle diameters measured by the different techniques, including the values derived from sedimentation FFF retention. There is good agreement between the electron microscope values and those derived from sedimentation FFF for the two particles of small sizes, and also between the values reported by Dow and the corrected sedimentation FFF value for their beads. The only instance in which the sedimentation FFF value is not confirmed by at least one other value is for the Polyscience nominal 0.358- $\mu\text{m}$  beads.

TABLE I

## POLYSTYRENE BEAD DIAMETERS OBTAINED IN VARIOUS WAYS

The sedimentation FFF values are an average of 4-6 points obtained under varying conditions.

Source	Reported diameter ( $\mu\text{m}$ )	Electron microscope diameter ( $\mu\text{m}$ )	Sedimentation FFF diameter ( $\mu\text{m}$ )
Polyscience	0.183	0.24	0.231 $\pm$ 0.007
Polyscience	0.275	0.31	0.323 $\pm$ 0.005
Polyscience	0.358	0.44	0.601* $\pm$ 0.025
			0.541 $\pm$ 0.010
Dow	0.357	—	0.368* $\pm$ 0.004
			0.356 $\pm$ 0.003

\* Corrected for finite size effects.

Clearly, the nominal 0.358- $\mu\text{m}$  beads give the most puzzling results. These are the largest particles and are therefore those for which finite size effects are most significant. We have not previously studied in detail conditions under which finite size effects are important, so our theoretical correction in this instance does not have the same proven validity as otherwise exists<sup>15</sup>. Nonetheless, the discrepancy is surprising because the sedimentation FFF results are reasonably consistent with one another over a 4-fold range of field strengths and flow-rates. The relative internal consistency of the results obtained under this range of conditions is shown in Table II. This consistency leads us to suspect some error in the electron microscope results, but this has not yet been checked.

As a final confirmation of the existence of errors somewhere in the values reported by the suppliers, we carried out a sedimentation FFF run on a mixture of the Dow nominal 0.357- $\mu\text{m}$  beads and the Polyscience nominal 0.358- $\mu\text{m}$  beads. If these diameters were correct, no separation would be possible and only a single peak would emerge. The resulting fractogram is shown in Fig. 4. Not only does separation occur, but a vacant interval lies between the peaks. While this reflects in part the high intrinsic resolving power of sedimentation FFF, it also demonstrates a striking error in at least one of the two reported bead sizes. The values in Table I suggest that the error resides with the Polyscience values.

TABLE II

APPARENT LATEX BEAD DIAMETERS,  $d$ , MEASURED BY SEDIMENTATION FFF FOR POLYSCIENCE NOMINAL 0.358- $\mu\text{m}$  PARTICLES

Run	Flow-rate (ml/h)	Field strength (gravities)	$R$	$l$ ( $\mu\text{m}$ )	$d$ ( $\mu\text{m}$ )	$d^*$ ( $\mu\text{m}$ )
A	18.2	63.0	0.0362	1.55	0.541	0.582
B	9.2	62.5	0.0346	1.50	0.549	0.596
C	18.5	124	0.0197	0.864	0.524	0.617
D	38.1	124	0.0186	0.813	0.535	0.642
E	38.1	62.5	0.0351	1.49	0.549	0.593
F	38.2	31.2	0.0685	3.00	0.549	0.574
Average					$0.541 \pm 0.010$	$0.601 \pm 0.025$

\* Corrected for finite size effects.

Returning to the matter of finite size effects in sedimentation FFF, we note that such effects increase not only with increasing particle size but also with increasing field strength and retention. Thus, at typical retention levels finite size effects are not important for particles of size up to *ca.* 1  $\mu\text{m}$ . However, there is an advantage to the high retention levels (low  $R$  values), such as those achieved here, where finite size

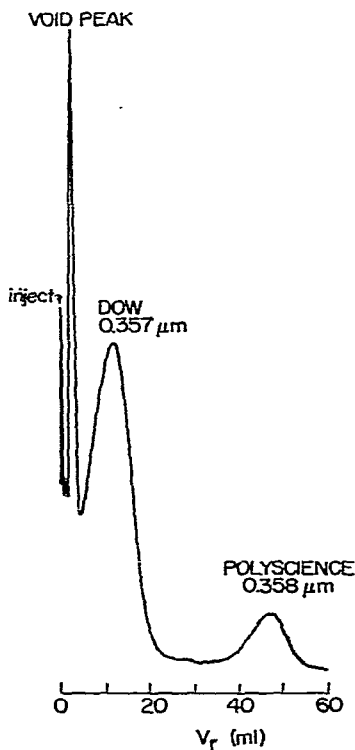


Fig. 4. Separation of Dow nominal 0.357- $\mu\text{m}$  polystyrene latex beads from Polyscience nominal 0.358- $\mu\text{m}$  beads by sedimentation FFF. The separation demonstrates the existence of an error in one of the reported bead sizes. Flow-rate, 38.1 ml/h; field strength, 36.1 gravities.

effects are most pronounced. This advantage is clear from the  $R$  dependence in eqn. 10. We can see the advantage intuitively by noting that the mean layer thickness,  $l$ , is a diffusion-equilibration distance, in some ways equivalent to the particle diameter of the packing in chromatography. We note from Table II that  $l$  values exist down to about  $0.8 \mu\text{m}$ , which permits rapid equilibration in the same way that small particles do in chromatographic packing. This factor is of great importance in dealing with the separation of fine particles because their diffusion is normally very sluggish, typically 2-4 orders of magnitude slower than that of small molecules.

We now proceed to show how the experimental conditions can be varied to control the retention, speed and resolution of a mixture of the three Polyscience latex beads.

#### *Retention and resolution control*

Fig. 5 shows the fractograms used to produce most of the retention data shown in Fig. 3, with the three polystyrene latex particle systems obtained from Polyscience. All of the fractograms are on the same time axis so that the results can be directly compared. The experimental conditions pertaining to each of the fractograms are given in Table III;  $\dot{V}$  is the channel flow-rate.

The shifting of peaks along the time axis in Fig. 5 has been accomplished not only by variations in flow-rate, but also by controlled variations in field strength. The latter control provides an important dimension to all FFF methods. With the aid of Fig. 5, we shall attempt to explain some of the important factors involved in field strength control.

We refer to the top fractogram in Fig. 5 as the reference run. Low-flow, high-flow, low-field and high-field are modifiers describing flow and field strength conditions relative to those used to obtain the top fractogram. All changes from the reference values of flow and field strength are approximately two-fold changes, either up or down. Thus a high-field run has approximately twice the field strength of the reference run, or about  $\sqrt{2}$  times the reference rpm's since the field strength increases with the square of rpm.

For clarity, it should be noted that the separation of particles with sizes lying as close to one another as those illustrated in Fig. 5, even those in the last two peaks of the fractograms, is difficult by techniques other than FFF. Clearly the separation can easily be achieved by sedimentation FFF, but the separation times are long. One could of course optimize for high speed, particularly with the second and third peaks, by various means including the offsetting of excess resolution against speed, following principles that are similar to those that have long been used in chromatography. To some extent this has been done in the lowest fractogram, F. However, our basic approach in this study was different: it was not so much directed at obtaining high speed as at showing how several important parameters influence speed, both positively and negatively. Once these relationships are broadly understood, optimization should follow simply.

We refer now to the changes observed with respect to the reference fractogram upon reducing the flow-rate by a factor of two. This change is shown in going from A to B in Fig. 5. As expected, all of the retention times are doubled, but the resolution of the first two peaks is simultaneously improved. This occurs exactly as in liquid or gas chromatography where a decreased velocity enhances resolution and plate

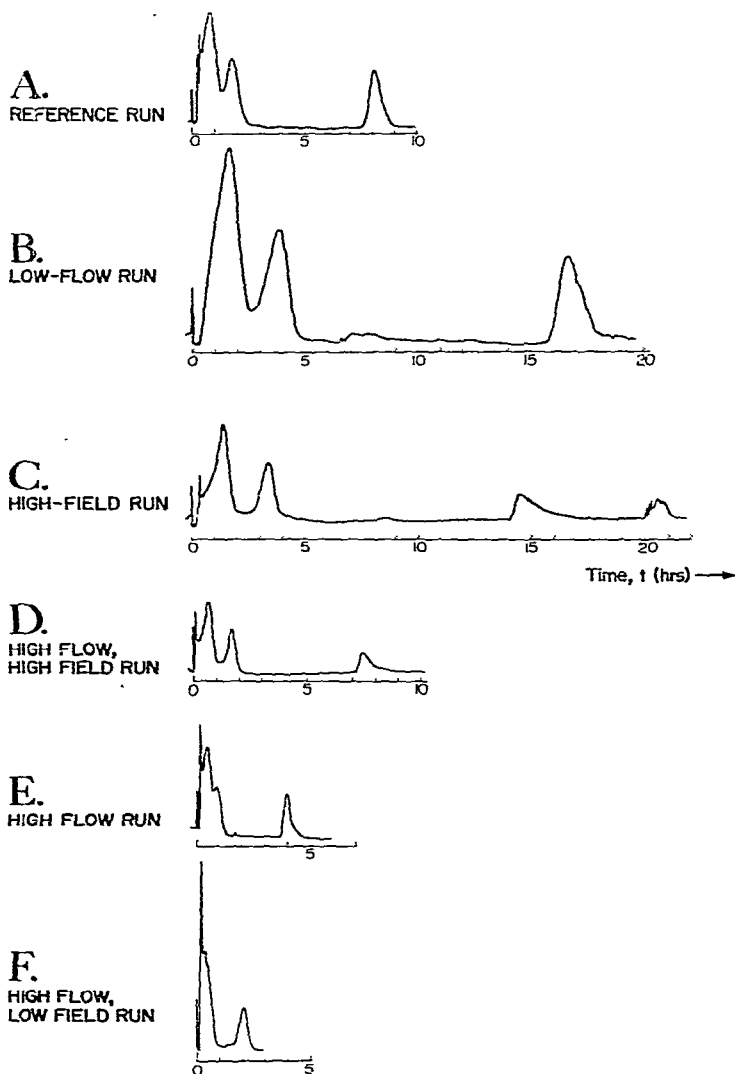


Fig. 5. Fractionation of the three sizes of Polyscience polystyrene latex beads by sedimentation FFF in a 0.9-m, 5.21-ml channel. Operating conditions are given in Table III. The reported bead diameters are 0.183, 0.275 and 0.358  $\mu\text{m}$ ; the diameters obtained from the peak positions in these fractograms are 0.231, 0.323 and 0.601  $\mu\text{m}$ , from left to right (see Table I).

numbers. In FFF the plate height is expected to vary linearly with velocity under ideal conditions where the middle term on the right-hand side in eqn. 9 is dominant. This is probably nearly true for the first two peaks, although measurements are difficult because of overlap. However, the final peak increases its plate count only from 1010 to 1150 in going from A to B. This result was at first puzzling, but we now believe that the width of the final peak is largely due to the polydispersity of the beads, despite the extremely narrow size range. If true, this would imply that the resolving power

TABLE III  
OPERATING CONDITIONS PERTAINING TO FIG. 5

Run	$\dot{V}$ (ml/h)	rpm	Field strength (gravities)	Stop-flow time (min)
A	18.2	852	63.0	30
B	9.2	849	62.5	30
C	18.5	1200	124.5	20
D	38.1	1200	124.5	20
E	38.1	849	62.5	30
F	38.2	600	31.2	45

and efficiency at high retentions are even greater than those suggested by the sharpness of the final peak. This matter is now under study.

In Fig. 5C, the field strength has been doubled with respect to its reference value. Again, the retention time (and in this instance retention volume, because the flow is at its reference level) is essentially doubled, in accord with eqn. 14, in which  $V_r$  is proportional to  $G$ , ignoring the factor  $1/3$ . However, the resolution of the first two peaks is superior to that in B, where the analysis time is roughly the same. This may be attributed to two factors. Firstly, resolution in all forms of FFF (as in all forms of chromatography) is intrinsically poor for peaks eluted near the void peak<sup>24</sup>. The increased field strength has increased the retention and thus the resolution of the first two peaks. Additionally, we noted earlier that plate height is expected to decrease rapidly with increasing retention. This, too, should contribute to the enhanced resolution of the early peaks in C.

The third peak in C is retained about 14.5 h, which, at a flow-rate of 18.5 ml/h, corresponds to a retention volume of 268 ml. This is roughly 51 void (column) volumes, a level of retention not achieved before in systems without programming. At this degree of retention, finite size effects are important, leading to elution earlier than expected. Consequently, the third peak in C is eluted earlier than that in B, in which, with a lower field, finite size effects are less important.

Most extraordinary is the small peak at the end of run C. We are aware of no contaminant in the system, yet the peak rises out of a baseline that has been consistently stable throughout these runs. Hence the peak possibly represents some unknown component which, judging by its compactness, may be monodisperse.

Runs B and C show that it is more efficient to increase resolution by increasing the field than by decreasing the flow-rate. If certain time restraints exist, then it is best to work under high field conditions, setting the flow-rate at whatever level satisfies the time requirements. Thus, if we are limited to the time taken in making the reference run, we would expect a better resolution at a higher field strength, and with the flow-rate increased enough to offset the additional time normally required for high-field runs. This principle is illustrated in run D, where such a strategy is seen to provide an improved resolution in a time slightly reduced from that of the reference run.

The sequence from D to F shows the general consequence of reducing the field strength at a constant flow-rate. The retention time decreases and so does the resolution. In E, the first two peaks have largely merged and in F they are essentially indistinguishable, yet the time is roughly halved for each two-fold reduction in the

field. If one has the object of separating the aggregate of the first two peaks from the last, the run is still successful at  $F$  and could be extended to even higher speeds.

The above discussion illustrates principles that are, in theory, valid for all FFF methods. It demonstrates that field strength is a most important dimension in retention, resolution and speed control in FFF. It is by no means a comprehensive discussion of optimization. Variables such as column dimensions have not been considered, and even the field strength has been dealt with in a limited way. It has been treated as a uniform parameter, thus excluding the versatility of control to be gained by varying the field during a run according to any number of possible programs.

Some of the additional aspects of optimization have been treated elsewhere, based on theoretical considerations<sup>13</sup>, but extensive efforts will be required in order to realize major goals of optimization.

#### FLOW FFF

Flow FFF requires that the channel walls be semi-permeable membranes<sup>7,26</sup>. A stream of solvent (disperse phase) is then forced through one wall and out of the other. The resulting cross flow is superimposed at right-angles on the axial flow and establishes a net motion of particles towards one wall, leading in a short time to a steady-state exponential distribution of a type that is typical of FFF systems.

The theory of flow FFF also derives from general FFF theory<sup>27</sup>. The quantity  $\lambda$  can be written as

$$\lambda = \frac{D V^0}{\dot{V}_c w^2} \quad (16)$$

where  $\dot{V}_c$  is volumetric rate of cross flow. As in sedimentation FFF, this can be substituted into the basic retention volume expressions (eqn. 6 or 7) to yield the theoretical retention equation. With eqn. 7 we obtain

$$\frac{V_r}{V^0} = \frac{\dot{V}_c w}{6 D V^0} + \frac{1}{3} \quad (17)$$

This equation shows that the retention volume,  $V_r$ , varies from one particle to another only by its dependence on the diffusion coefficient,  $D$ . The other parameters are related to the column dimensions and operating conditions, and remain constant in a run.

In order to establish a better relationship between  $V_r$  and particle size, we use the Stokes-Einstein equation:

$$D = kT/3\pi\eta d \quad (18)$$

where  $\eta$  is viscosity and  $d$  is the effective diameter or Stokes diameter of the particle. For spherical particles, the Stokes diameter is equal to the diameter of the sphere. When eqn. 18 is substituted into eqn. 17, we obtain

$$\frac{V_r}{V^0} = \frac{\dot{V}_c w}{2 V^0} \cdot \frac{\pi \eta d}{k T} + \frac{1}{3} \quad (19)$$

This shows that the retention volume is linear in Stokes diameter,  $d$ , just as in sedimentation FFF we find  $V_r$  to be linear in particle mass,  $m$ . Thus, using flow FFF and an appropriate detection system, one can obtain directly from the fractogram a linear particle size distribution curve beyond two void volumes. The cross-flow rate,  $\dot{V}_c$ , can always be adjusted to accommodate properly the particles under consideration; by this means, excessive retention volumes (times) can be avoided if the particles are large and, on the other hand, excessive elution within two void volumes can be avoided if the particles are small.

As with sedimentation FFF, flow FFF can, in theory, be used to characterize the dimensions of particles of a single size, or those of polydisperse systems. For instance, the diffusion coefficient,  $D$  (directly translatable to Stokes diameter,  $d$ , through eqn. 18), has been obtained for several viruses, although the existence of an apparent adsorption effect required the use of an empirical correction factor<sup>12</sup>. However, in general, using both proteins and polystyrene latex beads, good agreement with theory has been obtained without corrections<sup>27</sup>.

Flow FFF has also been used to obtain a particle size distribution for a polydisperse commercial paint pigment and to separate samples of silica colloids with a moderately narrow distribution<sup>25</sup>. An example of the latter is shown in Fig. 6 (data collected by Dr. Gwo-Chung Lin of our laboratories). The samples were provided by DuPont, and contain spherical particles of reported approximate diameters 0.012, 0.040, 0.065 and 0.13  $\mu\text{m}$ , from left to right in Fig. 6<sup>37</sup>.

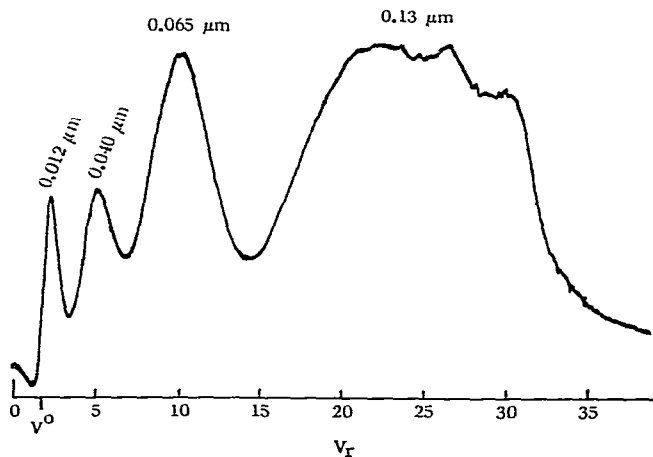


Fig. 6. Separation of DuPont colloidal silica beads by flow FFF. The reported particle sizes responsible for the peaks (left to right) are 0.012, 0.040, 0.065 and 0.13  $\mu\text{m}$ . Flow conditions:  $V_c = 10.8$  ml/h;  $\dot{V} = 3.1$  ml/h. Channel void volume,  $V^0 = 1.80$  ml.

All but the final peak in Fig. 6 are reasonably narrow and symmetrical, probably reflecting the finite polydispersity of the particles more than instrumental spreading<sup>25</sup>. Their elution volumes agree well with those predicted by theory, given the above particle sizes. The final peak has apparently suffered from a particle aggregation effect; it suggests that aggregation phenomena could be studied by flow FFF<sup>25</sup>.

The particles represented in Fig. 6 span a mass range exceeding 1000, much like the particles in Fig. 2, which were separated by sedimentation FFF. However, in the present instance the separation was achieved without benefit of programming. This illustrates the advantage that the intrinsic range of flow FFF is greater than that of sedimentation FFF. Also, we have been able to include particles that are so small ( $d = 0.012 \mu\text{m}$ ) that they would strain the capability of our present sedimentation FFF system, and this is by no means the lower limit of the range of flow FFF systems.

The above comparison illustrates the complementary nature of different FFF sub-techniques. Each has unique advantages that make it intrinsically more suitable than the others for certain applications. Taken together, the FFF sub-techniques yield an overall FFF methodology that has unparalleled scope in the analysis of complex particulate systems.

#### ACKNOWLEDGEMENT

This research was supported by Public Health Service Grant GM10851-20 from the National Institutes of Health.

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