Field-Flow Fractionation: A Versatile Technology for Particle Characterization in the Size Range $10^{-3}$ to $10^2$ Micrometers†

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Abstract

This article provides an overview on the use of field-flow fractionation (FFF) for particle size analysis and for the characterization of other particle properties such as density, porosity, and the thickness of adsorbed layers. While FFF is a relatively new technology for particle characterization, it is one of the most versatile and powerful techniques now available for characterizing particle populations. The unique features contributing to the effectiveness of FFF include high resolution, relatively high speed, adaptability to different types and sizes of particles, and the ability to collect narrow fractions for further characterization by microscopy and other techniques.

For background, the mechanism of FFF is described in two parts, one applicable to particles over 1 \( \mu \)m diameter and the other relevant to submicron size particles ranging down to 1 nm size. It is shown how particle size distributions are obtained for a variety of particulate materials in both size ranges. The strategies needed for measuring particle properties other than size and size distribution are discussed.

1. Introduction

Field-flow fractionation (FFF) is a family of techniques that affords a unique capability for characterizing populations of particles \(^{1-11}\). The methodology works by separating particle samples into narrow fractions for which various properties can be immediately deduced. The fractions can be readily collected and further characterized by a host of supplementary techniques including optical and electron microscopy, photon correlation spectroscopy, inductively coupled plasma-mass spectroscopy, and other chemical and physical techniques. The properties that can be obtained for the individual fractions by FFF measurements alone include, depending upon the experimental circumstances, particle mass, size, density, charge, porosity, shell thickness (for hollow and layered particles), and the thickness of adsorbed films. When the properties measured for the fractions are assembled for the entire particle population, the distributions and averages of the above properties are obtained. Among these distributions is the size distribution curve, which can be acquired at higher resolution levels than generally offered by other techniques, thus revealing finer details of the distribution.

FFF is a small-scale elution technique in which a narrow plug (generally 10-100 \( \mu \)L) of suspended sample is injected into a flow stream of carrier liquid (which can be aqueous or nonaqueous) that sweeps the sample into a special FFF channel within which separation takes place. The separated fractions emerge at different times from the outlet end of the channel and pass through a detector (usually a turbidimetric sensor)
FFF is used to measure the relative concentrations of the separated fractions. Since the retention (passage) time in the channel can be correlated with particle properties (see Section 3), the detector signal at any given time indicates the relative amount of particulate material having a specific value of the relevant property, which may be size, mass, or some other particle characteristic as described above. A plot of detector signal versus time—called the fractogram—then provides the information necessary to generate the corresponding distribution curve. The final step involves the use of computer software that converts the experimental fractogram into the desired distribution curve.

If the information provided by the fractogram is not sufficient to fully characterize the particle population, or if any doubt exists about the meaning of the data output, then particle fractions can be readily isolated by a fraction collector as they emerge from the detector. These fractions can then be subjected to virtually any other characterization technique (such as microscopy) capable of examining small samples of particles. None of the conventional particle sizing techniques allows such simple interfacing with other techniques to clarify, refine, and expand the information available pertaining to particulate samples.

Because FFF works with small injected samples, its uses are more analytical than preparative. The reduction in sample amount (to enable, for example, the characterization of microgram or nanogram quantities) is limited only by the detector sensitivity, while the increase in sample amount (for preparative purposes) is limited by various overloading phenomena to somewhere in the milligram range. For preparative operation in the gram and kilogram range, a high resolution technique related to FFF known as continuous SPLITT fractionation (CSF) has been developed.

We note that the FFF process can be readily automated by using an autoinjector to introduce successive samples into the system. Repetitive operation is then controlled and data analysis performed by computer.

The high resolution and high speed of one FFF subtechnique (termed sedimentation/steric FFF) applied to micron-size particles is illustrated in Fig. 1. Here the fractogram is shown for a mixture of seven monodisperse latex microsphere standards ranging from 4-29 \( \mu \text{m} \) diameter. The complete resolution of these particles is achieved in a time of less than 3 minutes. Each specific particle diameter produces a separate peak as can be quickly verified by microscopy.

Most particulate materials do not consist of a few discrete sizes as observed for latex standards; rather they are continuous distributions that produce broad continuous fractograms. However, the running of latex standards is invaluable in assessing the resolution of any particle sizing instrumentation. Any technique that cannot resolve latex standards because of inadequate resolution will almost inevitably broaden and
distort the true size distribution curve of the sample of interest. Such low resolution techniques care at risk of missing extra modes and other features of the distribution curve that are important in governing product quality.

For FFF, resolution can also be evaluated by examining the micrographs of collected fractions (see later). However, since no such separation and collection capability is found for most techniques, their resolution is difficult to determine except by using artificially narrow particle standards such as the latex standards.

2. Mechanism of FFF

Once particles enter the FFF channel they travel at different velocities along the channel axis and thus emerge at different times. For particle characterization it is important to understand (a) the mechanism of separation and (b) the relationship between particle properties and particle retention time, a relationship needed to obtain size distribution and other information.

The FFF channel is a thin ribbonlike channel as illustrated in Fig. 2a. The particle pulse enters the channel and is carried along its length by the flowstream. However, the velocity of any given particle depends upon the velocity of the flow lamina that it occupies. By applying an external field across the face of the channel such that the field extends over the channel's thin dimension, particles having different characteristics can be driven into different laminae where they assume unequal velocities and are thus separated.

The detailed mechanism of one form of FFF is illustrated in Fig. 2b, which shows a blown-up view of the channel interior and the two major confining walls. The parabolic flow profile set up between the walls is also illustrated. Different flow laminae have different velocities (as indicated by the arrows) depending upon their position along the parabolic curve. For example, any particle located near the center of the channel will be carried rapidly by the high velocity flow at the apex of the parabolic profile. A particle close to the wall will be carried much more slowly because the fluid velocity diminishes as the wall is approached. The applied field serves the purpose of driving particles with different characteristics into different laminar positions where they suffer different displacement velocities.

Figure 2b shows how this process works for the subtechnique of sedimentation/steric FFF (Sd/StFFF), which was used to produce the fractogram of Fig. 1. Here micron-size particles are driven by the field (in the case of Fig. 1 a sedimentation field) toward the accumulation wall of the channel. Although the particles are driven toward the wall, they do not quite touch its surface because shear-induced hydrodynamic lift forces oppose the driving force of the field and keep the particles elevated somewhat above the wall. Because of their smaller sizes, the centers of the smaller particles approach the wall more closely than do those of the larger particles. The smaller particles, because they are caught up in the slower laminae near the wall, are thus displaced along the channel axis at a slower velocity than are the larger particles. The different velocities (illustrated by the arrows of...
different lengths in Fig. 2b) give rise to the separation.

The above mechanism, applicable generally in the size range 1-100 \( \mu \text{m} \) (and sometimes beyond), is termed the steric mechanism of FFF$^{16,17}$. As the lift forces increase relative to the driving forces and the particles become more highly elevated, the steric mode of operation gives way to the hyperlayer mode$^{16}$. However, the distinction between steric FFF and hyperlayer FFF is not important for present purposes and will not be further discussed.

Submicron size particles, subject to more severe Brownian motion, form a diffuse cloud (see Fig. 3) near the accumulation wall rather than a single unique position like the larger particles$^{15,16,81}$. The diffuse cloud, having an exponential concentration distribution, corresponds to the normal mode of FFF operation, which is applicable to particle diameters extending from 1 or 2\( \mu \text{m} \) down to 10 \( \lambda \text{m} \), or 1 nanometer. The elution order in the normal mode is inverted relative to that in the steric mode with the smallest particles eluting ahead of their larger counterparts.

In principle, the fields that are used to generate separation in FFF can be any influence or gradient that extends across the thin dimension of the channel and is capable of driving particles into different laminar positions$^{611}$. The fields that have been employed in the order of their frequency of use are sedimentation, thermal (consisting of a temperature gradient), crossflow, electrical, and magnetic. Other fields are possible. Each field or gradient generates a driving force based on a different property of the sample particles and thus yields a different kind of information about the particle population. In addition, each field generally requires its own instrumentation that must be customized specifically for use with that field.

For sedimentation FFF, the thin ribbonlike channel is coiled around the inside circumference of a centrifuge basket (see Section 4). The field strength is governed by the rotation rate of the basket-channel system.

In flow FFF, the channel is sandwiched between two highly polished copper bars, one heated and one cooled. The temperature gradient creates the desired driving force through the phenomenon of thermal diffusion.

In thermal FFF, the channel is sandwiched so permeable to the carrier fluid that the carrier can percolate into the channel from one wall, cause the displacement of particles to the opposite (accumulation) wall, then egress through the permeable accumulation wall. The transverse flow or crossflow so generated is superimposed on the normal channel flow that continues to sweep particles along the channel axis. Wall permeability is gained by constructing the channel walls of frit elements; for the accumulation wall, the frit is covered by an appropriate membrane (see Section 4).

Sedimentation FFF has been the most widely used technique for particle analysis, particularly in the submicron size range$^{17,19}$. Flow FFF, a newer technique, is rapidly gaining ground in particle size analysis$^{20}$. Thermal FFF has been used mainly for polymer analysis but recently it has shown a capability for separating particles both in aqueous and nonaqueous suspensions$^{21}$.

We note that the field used in FFF is oriented perpendicular to the direction of flow and thus to the axis of separation. This unique perpendicular orientation of the field relative to the separation axis distinguishes FFF from
more widely used methods such as sedimentation, elutriation, and electrophoresis. The perpendicular arrangement of these two displacement axes allows the field and the flow to be varied independently. This independent control provides great flexibility in achieving high speed and high resolution goals.

3. Relationship of Particle Properties to Retention Time

Particle properties are deduced from their retention time \( t_r \), which equals the time required to displace the particle through the channel. Thus it is essential to establish a relationship between \( t_r \) and particle properties such as size, mass and density. Because of the complicated role of hydrodynamic lift force in influencing the lamina occupied by a given subpopulation of particles, the \( t_r \)-property relationship in steric and hyperlayer FFF (applicable generally to diameter \( d \geq 1 \mu m \)) is established empirically. For the normal mode of operation (used primarily for \( d > 1 \mu m \)), the \( t_r \)-property relationship is established by theory.

While the capability of FFF for submicron size analysis is perhaps most unique and has been by far the most common application to date, FFF is also a powerful and versatile technology in the supramicron (\( d \geq 1 \mu m \)) size range. We will address steric FFF first, which is mainly applicable to \( d \geq 1 \mu m \).

When crossflow is the driving force (giving the subtechniques of flow/steric FFF or flow/hyperlayer FFF), \( t_r \) is related to particle diameter \( d \) by the empirical logarithmic relationship

\[
\log t_r = S_d \log d + \log t_{r, f} \text{(flow FFF)} \tag{1}
\]

where \( t_{r, f} \) is the retention time of a particle of unit diameter and \( S_d \) is the diameter-based selectivity for flow FFF (the subscript \( f \) designates flow), a term that describes the sensitivity of \( t_r \) to changes in \( d \) according to the definition

\[
S_d = \left| \frac{d \log t_r}{d \log d} \right| \tag{2}
\]

Particle retention in sedimentation/steric FFF is best described by a slightly more extended relationship

\[
\log t_r = -S_d \log d + S_d \log G \Delta \rho + \log t_0 \text{(sed. FFF)} \tag{3}
\]

where \( t_0 \) is again a calibration constant and \( S_d \) is the diameter-based selectivity defined as in Eq.(2), but in this case applicable to the sedimentation field. (Typically, \( S_d = 0.75 \) and \( S_d = 1.25 \).) The parameter \( S_d \) is the density-based selectivity and \( G \) is the field strength expressed as acceleration.

Particle retention under the influence of a sedimentation field depends not only on particle diameter but also upon particle density, which is accounted for by the second term on the right of Eq.(3) in which \( \Delta \rho \) is

\[
\Delta \rho = |\rho_v - \rho| \tag{4}
\]

where \( \rho_v \) is the particle density and \( \rho \) is the (buoyant) density of the liquid carrier.

Equations (1) and (3) show that the crossflow (Eq.(1)) and the sedimentation (Eq.(3)) driving forces are complementary to one another, relating \( t_r \) to different properties or combinations of properties. For flow FFF, Eq. (1) shows that \( t_r \) depends solely upon \( d \), which is a kind of hydrodynamic diameter. For sedimentation FFF, \( t_r \) depends upon both \( d \) and particle density \( \rho_p \). Thus the choice of techniques depends upon the information sought. For particles of uniform composition such that \( \Delta \rho \) is fixed, either technique will yield a size distribution. If the composition and \( \Delta \rho \) vary, flow FFF still yields a size distribution but sedimentation FFF yields a mixture of size and density data. While this may be a disadvantage for the sedimentation field, we have developed some strategies for obtaining density information from sedimentation FFF, which can make the latter technique advantageous if the acquisition of
such density information is important\textsuperscript{20}. The calibration constants in Eqs. (1) and (3), \( S_{d1}, S_{d2}, S_{d0}, t_r, \) and \( t_0, \) are fixed empirically. These calibration parameters and the corresponding calibration plots of Eqs. (1) and (3) can be most easily established by using latex particle standards. For example, the fractogram of Fig. 1 provides a series of \( t_r \) versus \( d \) values for sedimentation/ steric FFF. A calibration plot of \( \log t_r \) versus \( \log d \) based on the data of Fig. 1 is shown in Fig. 4. The plot provides a value (specifically 0.694) of \( S_{d0}, \) which is the negative slope. Measurements must be made at other values of field strength \( G \) to establish \( S_{d0}; \) if neither \( G \) nor \( \Delta \rho \) vary, then the center term on the right side of Eq. (3) can be absorbed into the final constant \( t_0. \) If a sample being characterized has a different density (and thus a different \( \Delta \rho \)) from that of the latex calibrant, an adjustment of the rpm to make the product \( G \Delta \rho \) equal for sample and calibrant will make possible the direct use of the latex calibration curve\textsuperscript{21}.

As mentioned above, the \( t_r \) property relationship for submicron size particles (using the normal operating mode of FFF) is governed by theoretical equations. Specifically, no matter what the field type, \( t_r \) is related to the force \( F \) exerted on a particle by the field in accordance with expression\textsuperscript{11-16, 18-20}

\[
F = 3 \pi \eta d U
\]

In this case \( d \) is the Stokes diameter of the particle, \( \eta \) is the carrier viscosity, and \( U \) is the velocity of crossflow. Generally \( U \) is expressed in terms of the volumetric flowrate \( V \) of the crossflow stream by the expression \( U = V / bL, \)
where \( b \) and \( L \) are the channel breadth and length, respectively.

4. FFF Instrumentation

FFF instrumentation for particle and polymer analysis has been under development in the University of Utah laboratories since the 1960s when the concept of FFF was first described\(^2\). This instrumentation was improved continuously in the 1970s and 1980s\(^1\). The first commercial apparatus was a DuPont (Wilmington, DE, USA) sedimentation FFF system first released in 1986. This system is no longer being actively marketed.

Commercial instrumentation patterned more directly after our laboratory systems includes a thermal FFF apparatus first made available by FFFractionation, Inc. (Salt Lake City, UT, USA), in 1987. This was followed by a sedimentation FFF instrument, first marketed by FFFractionation in 1988, and a flow FFF system, first available in 1991.

A photograph of the FFFractionation sedimentation FFF instrument (Model S101) is shown in Fig. 5.

Although the intent of the present article is to provide an overview of particle characterization by FFF, the experimental results presented below (with two exceptions) are new. It is therefore appropriate, both for perspective and for specificity, to further describe the instrumentation used to produce these results. Since both sedimentation FFF and flow FFF systems were used to acquire experimental data, these two systems (and their variations) will be described separately below.

Our laboratory sedimentation FFF systems (as well as the commercial version produced by FFFractionation) is based on achieving particle separation in a thin ribbonlike channel like that shown schematically in Fig. 2a. However, the channel, rather than being straight, is curved to fit around the inside of a centrifuge basket where it is clamped between rigid concentric rings. The rotation rate is controlled by computer. The carrier liquid enters and exits the channel by means of special rotary seals\(^2\). The sample (usually 10–50 \( \mu \)L in volume) can be injected either by an injection valve or by a syringe. Following separation in the channel, the fractions are eluted into a detector, usually consisting of a commercial HPLC UV detector serving as a turbidity sensor. The data are then collected and analyzed by a computer.

The radius of rotation of the channel is 15.1 cm for both the laboratory and the commercial systems. However, the channel dimensions (particularly the thickness) are varied somewhat (see below) to best suit the sample under investigation. For most of the results presented in this paper, the tip-to-tip channel length is \( L = 90 \text{ cm} \), the channel breadth \( b = 1.0 \text{ cm} \), and the channel thickness \( w = 127 \mu \text{m} \). The flowrate \( V \) through the channel was varied depending upon resolution and speed requirements.

Flow FFF also makes use of a ribbonlike channel, but in this case the channel is linear in form as represented in Fig. 2a. Two flowstreams, oriented perpendicular to one another, pass through the channel. One flowstream (channel flow) transports the separated sample down the length of the channel while the other (crossflow) provides the field. The cross and channel flowrates are regulated by adjusting a variable pressure restrictor that is connected to either the detector outlet or the
crossflow outlet. Sample injection and detection are accomplished in the same manner as that used in sedimentation FFF.

In flow FFF the channel is clamped between two rectangular blocks (usually made of Plexiglass). Inset into each block is a flat panel of ceramic frit. The panel in the top block ensures that a homogeneous flow enters and moves across the channel thickness while the panel in the bottom block provides a rigid permeable support for the semipermeable membrane that serves as the accumulation wall. The primary function of the membrane is to retain the sample particles within the channel. The choice of membrane is determined by physical characteristics, compatibility with the carrier, needs based on sample characteristics (e.g., particle diameter and surface chemistry), and pressure-drop considerations. Ultrafiltration membranes have often been used in flow FFF systems. These membranes may be considered as universal membranes because of their general suitability for all of the particle sizes commonly encountered as well as for large macromolecules. Microfiltration membranes possessing different pore sizes have also been employed. Membrane composition is important for the reduction of particle-membrane interactions (and thus particle adsorption).

Several flow FFF channels were used in this work. The dimensions of the channels and the membranes employed are reported in Table 1.

The dimensions of the different FFF channel systems reported above are seen to vary considerably. The channel length and breadth are primarily determined in each case by considerations that relate to the integrated operation of the entire instrumental system; these considerations will not be described here. The most fundamental and operationally significant dimension of the channel is its thickness \( w \). In recent work we have tended more and more toward thinner channels. For example, in sedimentation FFF, where a 254 \( \mu \text{m} \) channel has long been considered standard, we have recently been doing much of our work with 127 \( \mu \text{m} \) thick channels. (In FFF we have thermal reduced \( w \) in one case to 50 \( \mu \text{m} \).) The thinner channels are more difficult to fabricate at the high uniformity levels required. In addition, they must have smaller volumes in the associated connecting tubing and detector cells and they must not be used for particles that are so large that the channel can become clogged. The operable size range is also somewhat reduced by thinner channels; the thinner channels cannot separate particles at the small and large size extremes that can be fractionated by thicker channels, all other factors (including field strength) being equal.

Offsetting the disadvantages of thin channels are several advantages. At the same field strength and retention times, particles emerging from a 127 \( \mu \text{m} \) channel are four times more concentrated in the effluent stream (whose flowrate is reduced fourfold) than those exiting a 254 \( \mu \text{m} \) channel. This higher concentration improves detectability. In addition, the relaxation time—the time required for particles to approach their equilibrium distributions upon entering the channel—is reduced in proportion to the decrease in \( w \). There is also some reduction in possible perturbations caused by secondary flow. Finally, for the steric mode of FFF, small particles (down to and below 1 \( \mu \text{m} \)) can be eluted rapidly from the thinner channels without requiring extraordinary flowrates.

5. Size Distribution for Supramicron Size \((d > 1 \mu \text{m})\) Particles

The size distribution of particles exceeding 1 \( \mu \text{m} \) diameter can be measured by both sedimentation FFF and flow FFF. Measurements made by sedimentation FFF will be described first. For particles in this larger size range, the steric mode of operation (see Fig.2b) is used. The means of calibration, described briefly above, are provided in more detail in a recent publication.

Fig.6 shows a fractogram obtained for a polydisperse Geon 213 PVC sample, a low porosity blending resin produced by BF Goodrich Company. This fractogram was obtained at 530 rpm with a flowrate \( V \) of 6.01 mL/min. The broadness of the Geon peak, in
contrast to the sharpness of the peaks in Fig. 1, confirms that the sample is polydisperse. However, nearly any instrument will yield a broad featureless response function like that in Fig. 6 for a polydisperse sample. Unfortunately, for most techniques the authenticity of the response curve is difficult to confirm and the resolving power (generally unrelated to the number of channels specified for a method) cannot be readily ascertained for the sample, although it can estimated (as discussed previously) using monodisperse latex standards. However for FFF, as noted earlier, fractions can be collected and subjected to further examination to verify resolution and accuracy. For the Geon run shown in Fig. 6, a series of fractions were collected and subjected to microscopy. The position of collection of four such fractions (9, 12, 15, and 19) are shown on the fractogram of Fig. 6. The accompanying panels in Fig. 6 show the micrographs of these four fractions plus the micrograph of the original (unfractionated) sample. The micrographs make it clear that fractionation has been achieved as predicted. These fractions show that very little aggregation has occurred over the bulk of the particle size range. However, the examination of a fraction collected from the void peak (not shown) confirms the presence of a few large aggregated clusters.

In order to obtain a size distribution curve for the Geon sample, a proper calibration curve must be established. As shown in the last section, the product $G_{\Delta \rho}$ must be the same for the sample and the calibrant. Since the density of the porous Geon sample is greater than that of polystyrene (1.33 versus 1.050 g/mL), the calibration run for polystyrene must be carried out at 1327 rpm rather than the 530 rpm used for Geon. Since the polystyrene calibration run of Fig. 1 and its plot shown in Fig. 4 were originally obtained at 1327 rpm for the purpose of characterizing Geon, the calibration plot of Fig. 4 is already perfectly matched to the Geon run.

When the parameters of the calibration plot are combined with the digitized response data of the fractogram shown in Fig. 6, a size distribution curve is produced. This curve is shown in Fig. 7.

Particles of irregular shape can be characterized by sedimentation/steric FFF as well. As an example of this, Fig. 8 shows a fractogram obtained for an alumina powder. The micrographs obtained for fractions 5, 9, and 16 verify the fractionation of the alumina into distinct size classes. Since the alumina run was made at 284 rpm and $V = 14.45$ mL/min, the polystyrene latex calibration run (not shown) was carried out at 2200 rpm (based on an assumed alumina density of 3.97 g/mL) using the same flowrate. The latex calibration plot combined with the fractogram yielded the size distribution curve also shown in Fig. 8. A cumulative size distribution curve for the alumina is shown as well. We note that in exploratory work we have been able to fractionate needlelike particles by FFF. However, the necessary calibration procedures have not been worked out.

Similar results in fractionating and
characterizing particles can be obtained using flow FFF. For diameter $d > 1 \mu m$, flow FFF is usually operated in the hyperlayer (which resembles the steric) mode. Flow/hyperlayer FFF has a somewhat higher selectivity than $Sd/St$FFF, but without programming the range of particle diameters that can be achieved in a single run is rather limited, being only about tenfold. However, when field strength and flowrate conditions are changed, the range can be shifted up or down. Most of our $F1/Hy$FFF runs have been used for particles in the overall size interval from 0.5 to 50 $\mu m$. Applications in this range include latex particles, pollen grains, biological cells, and silica particles.

As suggested earlier, the breadth of the size window that can be selected for any given run can be increased by using programming. Programming, a technique in which the field strength and/or flowrate is changed during the run, is used frequently to expand the size range available for submicron size particles using FFF in the normal mode. The extension of programming technology to flow/hyperlayer FFF to similarly expand the available particle size window has only recently been achieved. In Fig.9 we illustrate the power of programmed flow/hyperlayer FFF in the fractionation in a single run of polystyrene latex particles in the size range from 1 to 48 $\mu m$.

Size calibration curves for flow/hyperlayer FFF are most readily obtained using polystyrene latex standards as described above for sedimentation/steric FFF. However, for the flow FFF method, retention is independent of density (compare Eqs. (1) and (3)) so that no density compensation procedure need be used. Thus both the sample run and the calibration run are carried out at the same field
strength.

Flow/hyperlayer FFF has been used for the rapid characterization of HPLC silica. Fig.10 shows the fractogram obtained for a nominal 5 μm diameter porous chromatographic silica using nonprogrammed flow/hyperlayer FFF. The diameter scale that has been superimposed on the fractogram was obtained from a polystyrene latex calibration run. The results show that this particular material is anomalously broad and likely not suitable for high resolution chromatography.

As before, fractions of the sample were collected and subjected to microscopy. Micrographs of various fractions are shown in the panels of Fig.10. The size distribution curve deduced for this silica based on the fractogram of Fig.10 and the calibration run is shown in Fig.11.

We note that reliable size distribution curves for porous particles in the size range of HPLC silica are difficult to obtain. Light scattering methods generally lack the necessary resolution for these often narrow distributions. Electrozone methods are subject to error because of conductivity through the pores. While a correction can be attempted to compensate for this effect, there is no simple way to account for changes in pore conduct-
activity from one particle population to another or even from one particle to another. The pore conductivity, in general, will be a complex function of both pore volume and of pore connectivity in the particle, both of which may vary considerably for different samples.

6. Size Distribution for Submicron Size (d<1μm) Particles

Particle populations with d<1-2μm can be characterized both by sedimentation FFF and flow FFF systems operating in the normal mode. The relationship between retention time t and particle diameter d, rather than being obtained from empirical calibration plots as above, is acquired from the theoretical expressions of Eqs. (5)-(8). For particles in the approximate size range 0.5-2 μm, these equations must be corrected for steric effects. Particle populations that span both the normal and steric FFF size range (e.g., those having a range 0.2-5 μm) are difficult to characterize.

In work at the Field-Flow Fractionation Research Center we have applied sedimentation FFF to the size analysis of many submicron size materials including numerous latexes and emulsions, clays, viruses, subcellular particles, metal particles, silver halides, pigments, silicas, and colloidal particles found in natural waters. Other research groups have developed a large number of additional applications.

The fractogram of a sample of colloidal zirconia is shown in Fig.12 a. (This fractogram, obtained using a 254 μm thick channel, was previously reported in the literature.) This fractogram is typical for single mode submicron particle populations. Multimodal populations show multiple peaks (see later).

The fractogram of Fig.12a can be converted by computer software into the size distribution curve of the submicron zirconia. This distribution is shown in Fig.12b. If any question should arise about any aspect of this distribution (e.g., what fraction of particles in a certain size range are simply aggregates of the smaller particles), fractions can be collected and electron microscopy applied to more fully characterize the material.

The minimum particle size that can be characterized by FFF depends upon both the type of field used and its strength. Particles that are...
below the effective range of operation are poorly resolved from one another although they can be well resolved from larger particles that fall within the working size range. Although there is no sharp cutoff for the minimum resolvable size, in general the resolution is seriously hampered when the driving force \( F \) falls below the level

\[
F < \frac{6kT}{w} \tag{9}
\]

For sedimentation FFF a combination of Eqs. (7) and (9) shows that the minimum resolvable particle diameter \( d_{\text{min}} \) is approximated by

\[
d_{\text{min}} = \left( \frac{36kT}{\pi w G \Delta \rho} \right)^{1/3} \tag{10}
\]

Plots of \( d_{\text{min}} \) versus \( G \Delta \rho \) are shown in Fig.13 for two channel thicknesses (\( w = 127 \) and 254 \( \mu \)m) that are found to be highly practical for sedimentation FFF operation. The calculations underlying these plots are based on \( T = 298 \) K. The plots show that for a field strength \( G = 10^1 \) gravities (approximately the upper limit of our laboratory equipment and the FFF fractionation instrument) \( d_{\text{min}} \) values are about 0.03 \( \mu \)m for \( \Delta \rho = 1 \) g/mL. Lower and higher values of \( d_{\text{min}} \) are found for particles of higher and lower densities, respectively. To analyze much smaller particles, it is necessary to go to higher \( G \) values or to utilize flow FFF.

Flow FFF operates similarly to sedimentation FFF but its capabilities are somewhat different. Flow FFF, as noted earlier, gives density-independent particle size results, unlike sedimentation FFF. The resolution achievable by flow FFF is not quite as high as that of sedimentation FFF but it is generally much better than that of non-FFF systems, and thus adequate for most practical purposes (see Fig.14). Most importantly, flow FFF can be applied to particles of considerably smaller diameter than sedimentation FFF.

**Fig.13.** Plots of the minimum particle diameter (\( d_{\text{min}} \)) that can be resolved using the normal mode of sedimentation FFF operated at different levels of field strength \( G \) (in gravities) and different density difference \( \Delta \rho \) (expressed in g/cm\(^3\)). The two plots correspond to two different channel thicknesses (as shown) that have been found effective for sedimentation FFF operation.

**Fig.14.** The resolution of submicron polystyrene latex beads by flow FFF (shown here) is somewhat inferior to that of sedimentation FFF but is higher than that of other techniques in this size range. The fractogram shown here was obtained from flow channel III at a channel flowrate \( \dot{V} = 4.20 \) and a cross flowrate \( \dot{V}_c = 2.74 \) mL/min.
By combining Eqs. (8) and (9) we find that the minimum diameter resolvable by flow FFF is given approximately by the equation

\[ d_{\text{min}} = \frac{2kT}{\pi w \gamma U} \]  

(11)

This equation shows that for a modest crossflow velocity \( U = 10^{-1} \text{ cm/s} \) in a channel of thickness \( w = 254 \mu\text{m} \), the minimum resolvable diameter is \( 10^{-7} \text{ cm or } 1 \text{ nm} \). Thus flow FFF is not only a practical technique for the separation of particles in the common 0.1-0.5 \( \mu\text{m} \) size range, but it extends to diameters as much as 100 times smaller and has thus been found applicable in our laboratories to the separation of fine grained materials such as proteins, lipoproteins, humic acids, viruses, seed latex, carbon black, and small colloidal silica particles.

In Fig. 15 we show the flow FFF fractogram obtained for small polystyrene latexes in experimental flow channel IV (see Table 1) with \( V = 4.06 \) and \( V_c = 3.27 \text{ mL/min} \). These particles were sent to us by a major supplier of latex standards who was unable to characterize (even by electron microscopy) these smaller latexes. Although the fractograms could be converted into size distribution curves by the procedures noted above, we have elected instead in Fig. 15 to simply superimpose on the diagram a diameter scale calculated from the theoretical expressions, namely Eqs. (5) and (8). The positions of the peaks relative to the diameters scale show that the mean diameters range from \( 9-18 \) nm.

We noted before that a multimodal particle distribution will generally produce a multimodal fractogram. This is illustrated in Fig. 16 for an industrial styrene-butadiene latex. When the bimodal fractogram is converted into a size distribution curve (Fig. 16b), it produces a bimodal distribution. Note that by using flow FFF instead of sedimentation FFF on this material it is not necessary to know the latex density. On the
other hand, density values cannot be measured by flow FFF whereas numerous mass and density related parameters can be measured using a sedimentation driving force. This capability will be explained in the following section.

7. Other Particle Characteristics Measured by FFF

As noted earlier, in addition to measuring particle size distributions by FFF, we have employed FFF techniques for the measurement of a variety of particle properties, including particle density, porosity, aggregation kinetics, thickness of adsorbed layers, diffusion coefficients, thermal diffusivities, and polydispersities of narrow distributions. In addition, prospects exist for measuring shape factors and their distribution by FFF. It should be possible to extend our size and density measurements to a capability for simultaneously measuring the size and density distributions of complex particle populations such as those commonly found in environmental and biological samples.

The capability of FFF for providing a broader base of information than conventional techniques on particle populations is a consequence of several features of FFF operation. Among these enabling features are: (a) FFF is basically a high resolution separation methodology; (b) narrow fractions can be readily collected and further characterized by other techniques including other FFF methods; (c) either fundamental theory or empirical calibration can be used to relate particle properties to observed retention times; and (d) a number of different FFF techniques based on different fields are sensitive to different particle properties and can be used to deduce those properties.

Several examples of the measurement of properties other than size are described briefly below. Most of these measurements apply to submicron size particles but we will also describe a technique used for measuring the density (and thus porosity) of larger particles as well.

To begin with, we note that the retention time in sedimentation FFF (operating in the nomal mode) is a function of both particle mass $m$ and particle density $\rho_p$, (along with the carrier density $\rho_c$). The nature of this dependency is shown by Eqs. (5) and (6). If all densities are known, then the particle mass can be directly related to retention time $t$. This observation constitutes the basis for obtaining size and size distribution data as described above (see Eq. (7) and Section 6). However, the $t$-particle mass relationship can be utilized for a variety of particle characterization problems, a few examples of which are noted below.

If the carrier medium in which the particles are suspended also contains chemical substances that tend to adsorb on the particle surface, the particle will gradually accumulate an adsorbed film of the substance. This film adds to the particle mass and will thus cause a shift in the retention time. The amount of added mass can be calculated precisely using Eq. (5) and a modification of Eq. (6) that accounts for the film mass. By measuring the mass adsorbed at different concentrations of the dissolved substance, an adsorption isotherm can be obtained. The sensitivity of this method is enhanced if the density of the adsorbed surface film is greater than that of the particle. An example is the adsorption of protein on latex particles, a subject of considerable interest in the clinical industry. We have shown in a recent study that adsorbed layers of proteins and other substances having an average thickness as low as 1 Å is measurable by sedimentation FFF. A parallel study on the adsorption of water soluble polymers was carried out by Caldwell et al.

In the above study it was found that the exposure of polystyrene latex microspheres to human $\gamma$-globulin led not only to the adsorption of thin films (with average thickness 5-20 Å) of $\gamma$-globulin (from which adsorption isotherms could be determined), it also caused the low order aggregation of the latex beads. Aggregation, of course, causes a stepwise increase in particle mass and is thus readily measured by sedimentation FFF. Thus the low order aggregation of mono-
Disperse particle populations will generate a series of peaks in the fractogram, one for the single unaggregated particles (the singlets), another for clusters of two (the doublets), and other separable peaks for the higher order clusters. The area of each peak will represent the relative population of the corresponding particle cluster. Thus the above latex after 106 hours exposure to γ-globulin yielded substantial doublet and triplet populations as deduced immediately from the multimodal shape of the fractogram.

The ability to isolate and detect different sized clusters in aggregated materials has made it possible to follow the detailed kinetics of cluster formation and disruption using polymethylmethacrylate (PMMA) latex as a model colloid. For more complex colloids, a combination of sedimentation FFF and electron microscopy would be needed for a detailed analysis of the distribution of aggregated clusters.

Not only can FFF isolate clusters of different aggregation number \( n \) ( \( n = \) number of elementary particles in the cluster), its sensitivity is such that it can distinguish small differences between clusters of equal \( n \) that reflect unusual features of the particle population and, in some cases, of its origin. Thus in a recent study of 0.586 \( \mu \text{m} \) PMMA latex by sedimentation FFF, two doublet peaks were observed. One was retained about 10% longer than the other, showing (see Eqs. (5) and (6)) that the particles in that peak had a mass approximately 10% greater than that of the particles in the earlier doublet peak. Electron microscopy of the collected fractions showed that the particles appearing in the earlier peak were more tightly fused together than the doublets eluting later. An annealing mechanism was ruled out because the FFF results clearly demonstrated that the doublets differed 10% in mass, whereas no mass change would accompany annealing. The results were consistent with a two-stage latex formation process. The fused doublets apparently first formed by aggregation after the first stage of growth and subsequently continued to grow through the second stage, finally yielding the tightly fused doublets. These doublets have a smaller mass than those initially formed after the second stage of growth because the new growth was excluded around the contact point between the two spheres. The mass difference of 10\% could be used to calculate the diameter of the first stage latex, which was 0.3 \( \mu \text{m} \). This result was consistent with the measurement of the dimensions of the two doublets based on EM.

We note that the unusual features of this latex could probably be deduced from meticulous EM studies. However, a routine EM examination would suggest nothing unusual about this latex whereas the appearance of a second doublet peak in the fractogram of a sedimentation FFF run draws immediate attention to the unusual features described above. (EM remains invaluable as a complementary tool to FFF for working out geometrical details.) Thus FFF, because of its high resolution, provides not only detailed information about particle populations but transparent information whose major and minor features stand out. Such detailed features are becoming more important as the manufacturing, processing, and utilization of particulate materials is subject to increasingly demanding requirements.

Differences in particle mass can be discerned, measured, and interpreted as described in the above examples providing particle densities are known. If particle densities are unknown, then a single retention measurement cannot distinguish between mass and density effects since they jointly govern retention (see Eqs. (5) and (6)). However, if several runs are made with carriers of different density \( \rho \), the particle mass (or diameter) and density can both be determined. A special plotting procedure has been devised for this purpose. In recent work it has been shown that latex particle densities can be determined with extraordinary accuracy (to about 0.002 g/mL) using this sedimentation FFF procedure. The procedure works best when applied to particles that are close to the density of the carrier (usually water) but approximate densities have been obtained for particles as diverse as quartz and viruses.
Similar procedures for determining the density of large particles (>1 μm) by steric FFF are under development. However, a procedure has already been devised in which the density of supramicron size particles can be determined by a combination of the measurement of FFF retention and the determination of the mean particle diameter of the FFF fraction by microscopy. This procedure has been used to determine the density (and thus the porosity) of chromatographic support materials. It was used to determine that the density of the Geon 213 sample described in Fig. 6 and 7 has a value of 1.33 g/mL.

Since retention in flow FFF lacks a dependence on particle density, no density information can be obtained using this method. However, retention in the normal mode of flow FFF depends directly upon the diffusion coefficient $D$, thus making it possible to obtain $D$ values (and the associated Stokes diameters) from flow FFF retention measurements.

One of the most profound challenges in particle characterization is that of characterizing particle populations that exhibit significant variations in two independent particle properties such as size and density. These complex populations are common in most foodstuffs, soils, and environmental materials, as well as in many industrial products (e.g., cement). A particle size distribution does not fully characterize these materials. They would be far better characterized by a two-dimensional distribution in which size and density (the latter reflecting chemical composition) are the independent coordinates. Since size and density parameters can both be obtained from sedimentation FFF, it appears feasible to construct two-dimensional size-density “maps” of particle populations based on sedimentation FFF data. However, it is our belief that a more productive route involves a coupling of sedimentation FFF with flow FFF. We are currently working out the methodology for combining these two FFF techniques with the goal of providing a far more detailed means than presently exists for examining the detailed microfeatures of complex colloids.

The above strategy involves the separation and elution of fractions from one system and the shunting of these fractions into a second system to further break the subpopulation down according to the different properties governing fractionation in the second system. Since sedimentation and flow FFF have different dependencies on size and density, the experimental results can in principle be converted into the above mentioned two-dimensional distribution.

The capabilities and potential capabilities cited above constitute only limited examples of the scope of FFF in particle characterization. Since FFF is still a relatively youthful technology, its inherent strengths and versatility have not yet been widely adopted and exploited. Once these intrinsic capabilities are more broadly recognized, FFF can be expected to find a place as a major new technology for the increasingly demanding task of better characterizing the ubiquitous particulate materials of industry and of our everyday surroundings.

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Nomenclature

- $d$: particle diameter
- $d_{\min}$: minimum resolvable particle diameter
- $F$: force exerted on a particle by the field
- $G$: field strength expressed as acceleration
- $K$: Boltzmann constant
- $m$: particle mass
- $S_{fl}$: diameter-based selectivity for flow FFF
- $S_{sd}$: diameter-based selectivity for sedimentation FFF
- $S_{\Delta \rho}$: diameter-based selectivity
- $t_r$: retention time
- $t_c$: calibration constant
- $t_{11}$: retention time of particle of unit diameter
- $t_v$: channel void time
- $T$: temperature
- $V$: channel flowrate
- $V_c$: cross flowrate
- $w$: channel thickness
- $\eta$: carrier viscosity
- $\rho$: density of liquid carrier
- $\rho_p$: particle density
References