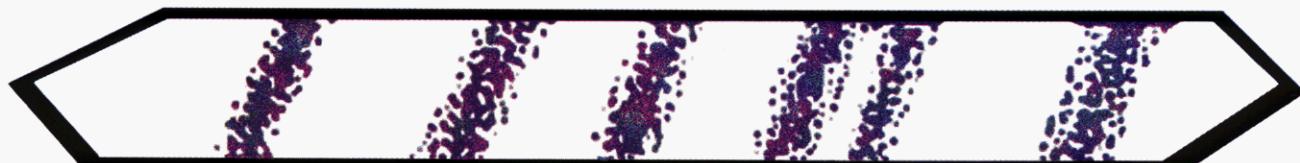


# Measuring Colloidal and



# Macromolecular Properties by FFF

**F**ield-flow fractionation (FFF) is a family of chromatographic-like elution techniques in which an external field or gradient, rather than partitioning between phases, causes differential retention. FFF techniques are applicable to a broad range of polymers, biological macromolecules, colloids, polymer-colloid complexes, and larger cell-sized particles.

FFF has two broad functions: separation and measurement (1). The separation process is best known; this article, however, describes FFF measurement. It enumerates various properties of macromolecules and colloids that can be measured by FFF but are often difficult or impossible to measure using other techniques. This article further explains how FFF measurements are made and how these measurement capabilities are enhanced by the separation process.

FFF separation originates in the fact that different field-based forces acting on unlike macromolecules or particles in an FFF system induce differential migra-

*FFF can be used to measure both primary and secondary properties of colloids and polymers*

tion. The measurement capabilities of FFF have a related origin: Quantitative values of the forces responsible for separation can be obtained from measured retention times. Measurement of these forces can provide the analytical chemist with a host of fundamental physicochemical constants for the retained particles and macromolecules.

Different constants are acquired using different fields (1, 2). By using a variety of fields, a wealth of fundamental physicochemical data can be acquired, including particle and polymer mass and molecular weight, density, equivalent spherical diameter, hydrodynamic diameter, charge, diffusion coefficient, and thermal diffusion coefficient.

The acquisition of a primary property, such as mass or molecular weight, aids in the characterization of numerous secondary properties, such as the mass of an adsorbed film on a colloidal particle, the shell thickness of a core-shell latex, the surface area of a lipid bilayer in a vesicle, the level of colloidal aggregation, or particle composition (Figure 1).

Measurement capability is enhanced by the fractionating power of FFF. Species can be isolated from complex matrices and simultaneously subjected to measurement. Thus the properties of a number of intermixed components can be measured in a single run (Figure 2). For populations having properties with a continuum of values, the property distribution can be measured, yielding mass distribution, size distribution, and so on.

The purified fractions in the eluting FFF stream can be diverted (often on line) to other instruments in which complementary measurements can be made fraction by fraction (1, 3–6), as shown in Figure 2. These measurements can be made using electron microscopy, light microscopy, photon correlation spectroscopy, classical light scattering, multiangle laser light scattering, ICPMS, or another FFF

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technique, and may yield either physical constants or chemical composition profiles (3). The property or properties measured by these downstream techniques, when combined with the property measured by FFF, enrich the information base that characterizes the sample and its components. Thus broad new capabilities have arisen for characterizing complex colloids of environmental, biological, and industrial origin.

Other separation techniques can be used for measuring macromolecular and particulate properties, but their scope is more limited, providing at most a single property. For example, size exclusion chromatography (SEC) yields the hydrodynamic diameter of polymers (7), but the measurement requires a calibration run with known standards, is based on the assumption that hydrodynamic diameter underlies retention rather than on theory, and cannot be readily extended to high molecular weight polymers or typical colloids.

In contrast to SEC, HPLC gives the distribution coefficient  $K$ , which yields information on relative hydrophobicity, polarity, and related properties. However,  $K$  values, which arise from the mutual interaction of a solute and a stationary phase, are difficult to translate into true physicochemical constants of the solute alone.

Capillary electrophoresis can yield electrophoretic mobilities if the electroosmotic flow is properly accounted for (8). Mobility, a useful parameter, is actually a composite of more fundamental parameters: effective charge and friction coefficient (9). Mobility values cannot be broken down into these fundamental constants without complementary measurements.

FFF, however, is capable of measuring a large array of properties, all based on simple theoretical principles. It is readily coupled to other instruments and can be used for the difficult but increasingly important range of large polymers, microgels, simple and complex colloids, various association complexes, and diverse particles with sizes from 1 nm to 100  $\mu\text{m}$ .

The recognition of measurement as a capability of FFF occurred early in its development. For example, in a 1975 paper (10), we stated, "The elution pattern in SFFF [sedimentation FFF] hinges on properties such as molecular weight, density, diffusion coefficients, and related quantities; one can thus obtain various physical parameters from measured elution properties." Since then, numerous studies have been reported on measurements made by FFF (1, 11).

### Basis of measurement

FFF is a differential elution process outwardly resembling chromatography but applicable across a much greater mass range. The retention of a component depends on one or more of the component's properties. A relevant property,  $p$ , of a macromolecule or colloidal particle can be calculated when a valid relationship exists between the property and the retention time  $t_r$ , or volume  $V_r$ . The simplicity of the FFF channel, with its open parallel-plate structure, leads to theoretical  $t_r-p$  relationships with only an occasional need to rely on calibration.

FFF takes place in a thin ribbonlike channel (Figure 2). A field or gradient applied perpendicular to the channel axis drives components toward one wall (the

accumulation wall) of the channel, where each forms a steady-state distribution. The compression of the distribution against the accumulation wall increases as the force applied by the field on the particles increases. Because of the different force levels, different components have steady-state distributions of different thicknesses. In most cases, particles are driven to within 1–10  $\mu\text{m}$  of the accumulation wall.

A stream of carrier liquid transports the particles toward the channel exit and a detector. The flow is laminar and parabolic because of channel thinness (75–250  $\mu\text{m}$ ). Components with distributions compressed tightly against the accumulation wall are carried slowly by flow because they are immersed in streamlines of low velocity adjacent to the wall. More expanded distributions (subject to lesser forces) travel more rapidly and elute earlier. If the channel structure is uniform with known dimensions and the field is applied evenly, the displacement velocity and thus the retention time of a component can be related by theory to the force exerted on the component particles. Calculation of the forces yields knowledge of particle properties (1, 2, 12).

In principle, any field or gradient capable of differentially compressing sample components against the accumulation wall

### Force equations

$$F(\text{sedimentation}) = m'G = mG(\Delta\rho/\rho_p) = V_p \Delta\rho G = (\pi/6)d^3 \Delta\rho G \quad (2)$$

$$F(\text{flow}) = fU = 3\pi\eta d_h U = (kT/D)U \quad (3)$$

$$F(\text{thermal}) = D_T f(dT/dx) = \alpha k(dT/dx) = kT(dT/dx)(D_T/D) \quad (4)$$

$$F(\text{electrical}) = qE = \mu fE \quad (5)$$

### Properties derivable from known forces

$m'$  = effective mass

$m$  = particle mass

$V_p$  = particle volume

$\Delta\rho$  = density difference

$\rho_p$  = particle density

$d$  = effective spherical diameter

$d_h$  = hydrodynamic diameter

$f$  = friction coefficient

$D$  = ordinary diffusion coefficient

$D_T$  = thermal diffusion coefficient

$\alpha$  = thermal diffusion factor

$q$  = effective charge

$\mu$  = electrophoretic mobility

**Other symbols:**  $dT/dx$  = temperature gradient,  $E$  = electrical field strength,  $G$  = acceleration,  $k$  = Boltzmann's constant,  $T$  = absolute temperature,  $U$  = crossflow velocity,  $\eta$  = viscosity

can be used in FFF. Because this compression is opposed only by Brownian motion, weak forces suffice, and many types of fields can be used. Each field exerts a force with its own unique dependence on particle properties and its own selectivity.

Four fields have been widely studied. Most prominent is sedimentation, usually generated by a centrifuge but sometimes by gravity. The sedimentation force acts perpendicularly to the flow-separation axis, a configuration with little resemblance to conventional centrifugation.

Other important fields include a temperature gradient (thermal FFF), in which the perpendicular force is a result of thermal diffusion; a cross flowstream of carrier liquid (flow FFF), in which the force originates in the friction of the cross flowstream moving across the components; and electrical fields (electrical FFF), in which force depends on particle charge.

The starting point for measuring macromolecular and colloidal properties is the so-called standard equation relating retention time  $t_r$  to the force  $F$  exerted by the applied field on a single particle. When the particle is strongly retained,  $t_r$  becomes proportional to  $F$ , as shown in the approximate form of the standard equation

$$t_r = \frac{Fwt^0}{6kT} \quad (1)$$

where  $T$  is absolute temperature,  $k$  is Boltzmann's constant,  $w$  is the channel thickness, and  $t^0$  is the channel void time (the elution time of a nonretained species). The standard equation provides the mathematical link needed to convert an experimentally measured  $t_r$  into a value for force  $F$ .

Once the force on a component is obtained, the next step is to translate this force into the desired properties of the component. Here we step outside the arena of FFF theory and use the classical scientific laws that relate forces to the properties of bodies on which the forces are exerted. Four classical force equations, for the four major fields of FFF, are shown in the box on the previous page. These equations show how  $F$  depends on various particle properties; the properties in these equations can be measured by FFF.

This approach applies to macromolecules and colloidal particles but is not applicable to particles (such as biological cells)  $> 1 \mu\text{m}$  in diameter. Larger particles are governed by a steric mechanism of separation, which alters the relationship between retention time and force; properties of larger particles (such as size, density, porosity, and hydrodynamic lift forces) can be measured by steric FFF (13, 14). These measurements will not be described here.

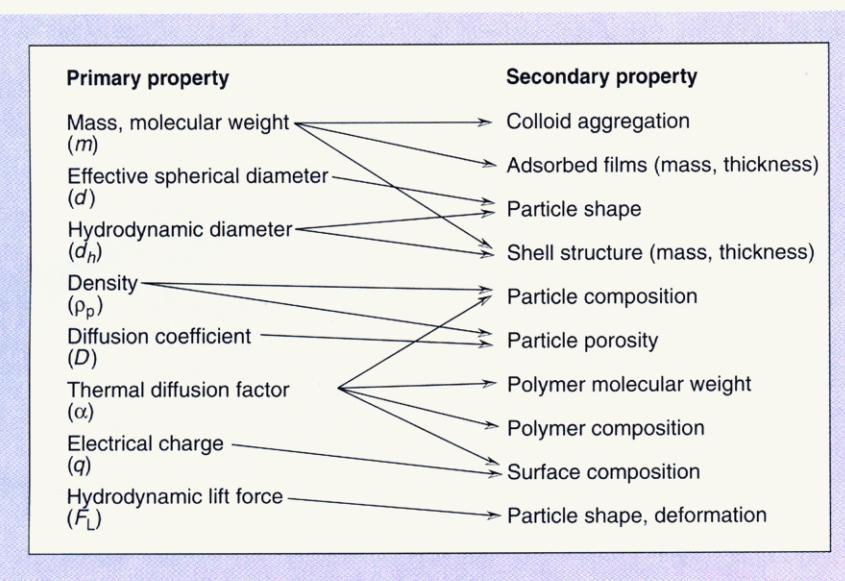
## Primary measurements

Primary properties of a retained particle or polymer are those that can be measured directly from FFF retention data with little or no prior knowledge about the species. (Primary properties generally appear in the basic force equations; the properties shown in the box are examples.) Secondary properties, by contrast, generally require outside information, assumptions, or a model describing the retained components (e.g., they are needle shaped, aggregated, coated by a thin film, or their composition is known). Note that a primary property measured by one FFF technique may become a secondary property when measured by another FFF technique.

**Flow FFF.** Flow FFF is one of the most universal separation techniques. It can separate and measure virtually all classes of macromolecules and particles in both aqueous and nonaqueous environments.

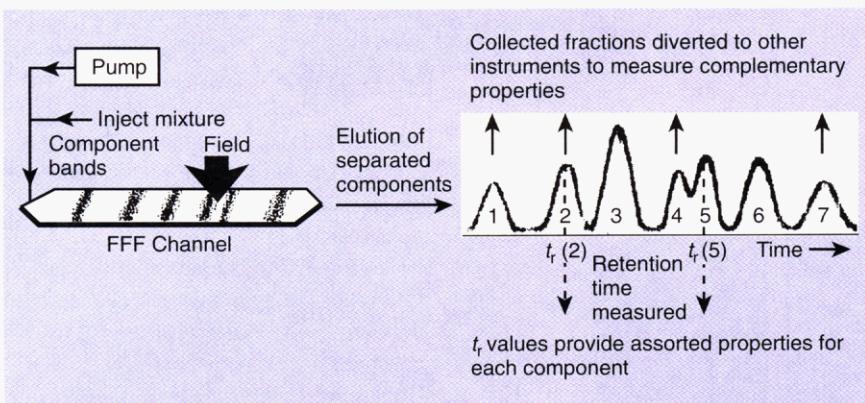
As shown in the box,  $F$  in flow FFF is related to several physical parameters of the particles or polymers: the hydrodynamic diameter  $d_h$ , the diffusion coefficient  $D$ , and the friction coefficient  $f$ . We consider these primary properties because they are obtained directly from flow FFF retention measurements without any need to know the composition or conformation of the retained components. Whether the retained species are polymeric, particulate, aggregated, associated, coated, or platelike is irrelevant to the measurement.

Figure 3 shows the flow FFF of two circular DNA molecules (one single stranded and the other double stranded) that have approximately the same chain length (15). The single-stranded DNA elutes first because it is more flexible and folds into a more compact random-coil structure with a smaller  $d_h$  and a larger  $D$  than the double-stranded DNA. Although some noise is apparent in the peaks because of the small amount injected ( $\sim 0.2 \mu\text{g}$  each), the retention time can be measured to within a few percent. Based on Equation 3 in the box, the ratio of retention times of the two peaks equals the ratio of the hydrodynamic diameters, 2.1:1. The two diffusion coefficients (reduced to  $20^\circ\text{C}$  in water) are calculated to be  $5.01 \times 10^{-8} \text{ cm}^2/\text{s}$  and  $2.44 \times 10^{-8} \text{ cm}^2/\text{s}$ . Diffusion co-



**Figure 1. Primary and secondary properties of colloids and macromolecules measurable by FFF.**

Arrows indicate routes (not comprehensive) from primary to secondary properties.



**Figure 2. Approaches to measuring various properties of macromolecular and colloidal/particulate mixtures using FFF.**

First, the measured retention time of any separated component can be converted to a fundamental component property (bottom) with different fields yielding different properties. Second, any desired component can be collected and subjected to measurement by some other instrument (top), leading to measurement of additional properties for that component.

efficients for other DNA molecules have been similarly obtained; the average standard deviation of the diffusion coefficients for all DNA molecules measured was 4.1% (15). We observe that although single- and double-stranded DNA was injected as a mixture, diffusion coefficients can be measured for both components as a result of the separation.

Many diffusion coefficients have been reported for proteins as well (16). Measurement precision is about 5%, comparable to that of other techniques. The flow FFF method for measuring protein diffusion coefficients is rapid (run times down to 1 min), can be automated, and can resolve important minor components such as protein dimers while simultaneously measuring their diffusion coefficients.

Because flow FFF yields a diffusion coefficient, hydrodynamic diameter, or friction coefficient for each retention time, continuous distributions yield a continuum of retention times and thus a continuum of diffusion coefficients, hydrodynamic diameters, or friction coefficients. As a result, size distributions can be calculated. Because of the technique's high resolving power, details of the size distribution are revealed with much greater fidelity than that provided by dynamic light scattering.

Some measurement uncertainty in flow FFF arises from the uneven surfaces and compressible membranes used as the accumulation wall of the FFF channel. Accuracy can be improved by using a calibra-

tion standard with a known diffusion coefficient or hydrodynamic diameter or by coupling the flow FFF system to an online detector, such as a multiangle laser light-scattering instrument, that can determine the size or molecular weight of each fraction. Measurements made by sedimentation FFF tend to be more accurate than those from flow FFF because of higher selectivity and more precisely machined channel walls.

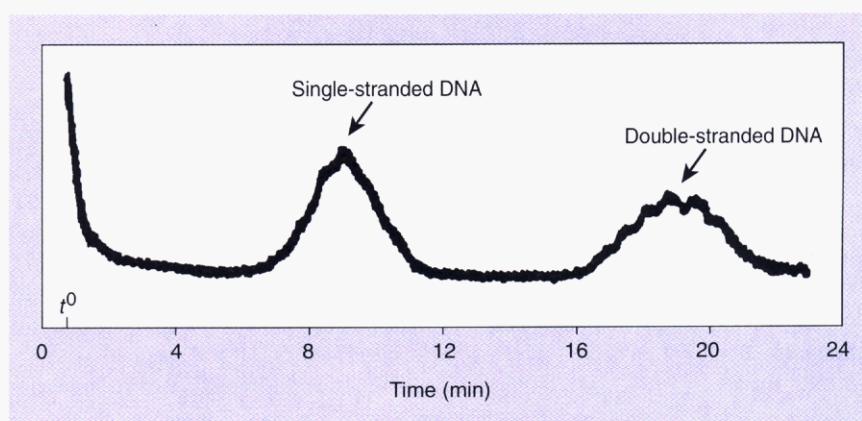
**Sedimentation FFF.** Sedimentation FFF is a powerful method for the separation and characterization of colloidal particles. Particle mass governs sedimentation forces, and is thus measurable by FFF. However, sedimentation forces are

complicated by buoyancy; the mass actually measured is the true particle mass minus the mass of liquid displaced by the particle. This mass difference is known as the effective mass  $m'$ , and it is  $m'$  that emerges most directly from sedimentation FFF measurements.

Although buoyancy complicates sedimentation FFF measurements, it also enriches measurement opportunities. For example, controlled variations in the density of the carrier liquid (e.g., by adding sucrose) will alter buoyancy forces by a known percentage and thereby produce data yielding true particle mass  $m$ , density  $\rho_p$ , volume  $V_p$ , and effective spherical diameter  $d$  (1, 11).

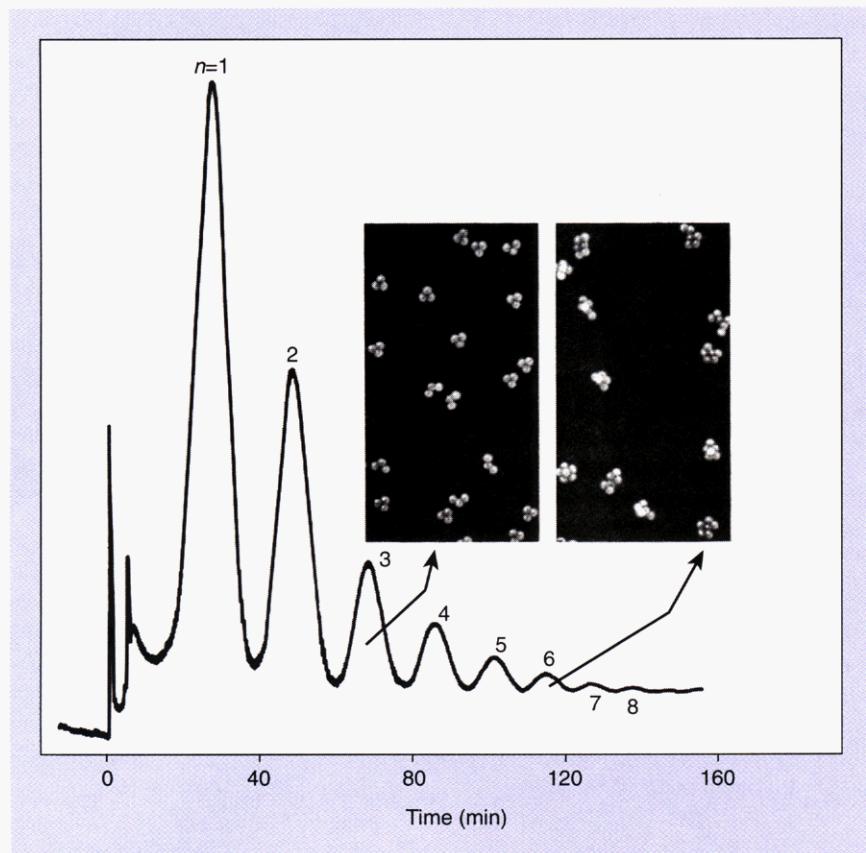
Using this approach, with carrier densities adjusted close to the particle density of latex, the actual density of latex particles and other low-density colloids can be determined to four-figure accuracy. The measurement is so sensitive that small effects due to molecular restructuring at the interface can be detected (17).

A shortcut is usually taken to measure particle diameter. If particle density is known, a single measurement of the force, rather than a series of measurements at different carrier densities, yields the effective spherical diameter. Technically, if a particle's density is required to measure its diameter, the diameter is a secondary property. (Density is never needed to acquire diameter in flow FFF, thus diameter from flow FFF is unequivocally a primary property.) Because particle densities



**Figure 3. Separation of single-stranded from double-stranded circular DNA chains of the same approximate length by flow FFF.**

Separation is based on differences in hydrodynamic diameter, or equivalently, diffusivities, both of which can be obtained in terms of measured retention times. The single-stranded DNA contains 7599 bases and has a molecular weight of 2,510,000; the double-stranded DNA contains 7250 base pairs and has a molecular weight of 4,785,000.



**Figure 4. Separation of aggregates of different cluster size by sedimentation FFF.**

The elementary particles forming clusters are monodisperse polymethylmethacrylate latex microspheres with  $0.23\text{ }\mu\text{m}$  diameter. Each succeeding peak corresponds to the addition of one elementary microsphere to the previous cluster. The total number of microspheres per cluster ( $n$ ) varies from 1 to 8. The fact that successive peaks are generated by pure populations of a fixed cluster size  $n$  is verified by electron microscopy of collected fractions. (Adapted with permission from Reference 24.)

are commonly known, we include for convenience particle diameters measured by sedimentation FFF in this section on measurement of primary properties.

For continuous particle distributions, sedimentation FFF, like flow FFF, yields high-resolution size distribution curves. However, size distributions, like single sizes, can be obtained from sedimentation FFF data only if particle density is known. For nonspherical particles, the diameters obtained by sedimentation FFF and flow FFF data are different, with sedimentation FFF providing equivalent spherical diameter and flow FFF providing hydrodynamic diameter (1).

**Thermal FFF.** Thermal FFF has been used primarily for fractionating polymers, especially those of high molecular weight. Recent studies have shown that thermal FFF is also applicable to particles, both in aqueous and nonaqueous media. For

both polymers and particles, retention depends on composition as well as size.

Thermal FFF, as shown by Equation 4 in the box, provides values for the thermal diffusion factor  $\alpha$  and the ratio of the thermal diffusion coefficient to the ordinary diffusion coefficient  $D_T/D$  for both polymers and particles. These parameters describe the strength of thermal diffusion, a poorly understood transport process that is important for physicochemical studies. More importantly,  $D_T/D$  can be converted into polymer molecular weights (18), a secondary measurement that will be discussed in the next section.

**Electrical FFF.** In principle, electrical FFF is capable of measuring the charge residing on macromolecular and colloidal species. Although implementation of charge measurements has been slow, recent advances have been made by Caldwell and co-workers (19).

## Secondary measurements

Secondary properties arise when primary properties measured for particles or polymers are extended to incorporate new features by virtue of some model, assumption, or body of prior knowledge. The possibilities for practical measurement are numerous.

**Polymer molecular weight.** Flow FFF yields the hydrodynamic diameter of polymers, particularly water-soluble polymers, as a primary property. If it is assumed, following random-coil models (20), that the hydrodynamic diameter is related to molecular weight by  $d_h = KM^n$ , molecular weight (and molecular weight distribution) can be obtained as a secondary property.

Similarly, thermal FFF yields the thermal diffusion factor or the ratio of the thermal diffusion coefficient to the ordinary diffusion coefficient as primary properties. Molecular weight is not a primary property. However, for linear polymers it has been established empirically that  $(D/D_T) = \phi_0 M^{-n}$ , where  $\phi_0$  and  $n$  are universal calibration constants (18). Once these constants are known for a given polymer–solvent system, the polymer molecular weight and molecular weight distribution can be determined.

If the diffusion coefficient for either polymers or particles can be independently determined, thermal FFF yields the physicochemical parameter  $D_T$ . Thermal FFF has thus become the simplest available tool for measuring  $D_T$ , and abundant data have been produced.

**Association and multiphase colloids.** Sedimentation FFF is the most powerful FFF technique for measuring the secondary properties of complex colloids, particularly of particles that consist of two or more parts or phases bonded together. For such particles, the mass (or effective mass) measured by sedimentation FFF is a simple sum of the mass of the individual parts. Several strategies are available for isolating constituent masses, thereby providing an analytical tool for unraveling the structure and composition of complex colloidal materials.

One strategy for two-phase particles is to adjust the carrier density so that one phase is neutrally buoyant; the other phase is then responsible for the measured force and its effective mass can be

calculated. For example, if the carrier density is adjusted to equal the density of a liposome's liquid interior, the interior mass effectively becomes invisible and the measured mass is reduced to that of the encircling lipid bilayer. Because the density and thickness of the bilayer can be estimated, the area of the bilayer can be obtained. For unilamellar vesicles, area can be translated into diameter. (An independent diameter can be measured by flow FFF.) For polydisperse populations, distributions in lipid bilayer mass, area, and vesicle size can be obtained (1, 21).

**Colloidal aggregates.** The formation of aggregates is one of the most important phenomena in colloid science (22, 23). The distribution of subunits in aggregated clusters is needed to understand aggregation dynamics. The measurement of cluster distributions is difficult, even by electron microscopy.

FFF techniques are highly sensitive to aggregation (5). Aggregated subpopulations elute later than do nonaggregated species because of the increased force. Sedimentation FFF is particularly informative about aggregation because the force is proportional to cluster mass and is additive for all subunits. When monodisperse particles aggregate, each size cluster generates its own peak, as shown in Figure 4 (24). The spacing of successive peaks is regular with peak area proportional to amount.

For polydisperse colloids, cluster peaks will overlap, yielding continuous mass distributions shifted to higher mass values than those of nonaggregated populations. A combination of sedimentation FFF and electron microscopy is required to unravel the aggregation. The equal-mass fractions collected from an FFF run can be further broken down into morphological classes by electron microscopy. The potential of these two complementary techniques is substantial (5).

**Particles with adsorbed layers or shells.** Particles with relatively homogeneous interiors but with an outer layer or shell of a distinctly different composition are major players in many fields, including industry, biology, pharmacy, and the environment. Such particles include liposomes, cells, core-shell latexes, immuno-diagnostic latexes, waterborne colloids, and a myriad of other particles in different

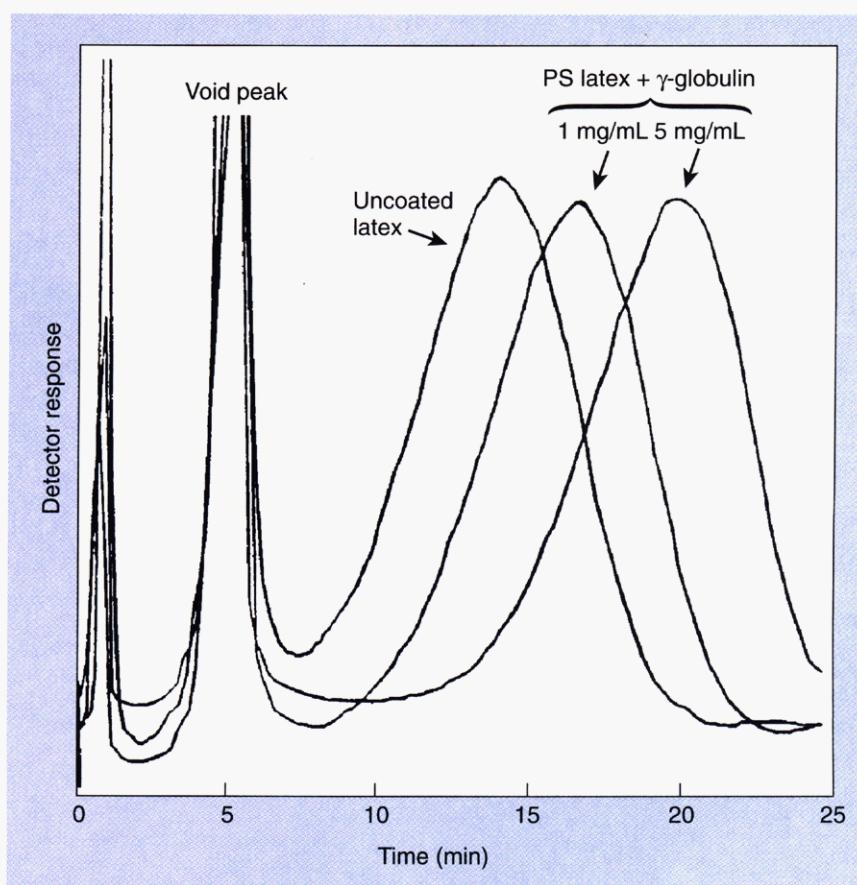
environments that have adsorbed some chemically or biologically active substances on their surfaces. Many environmental particles adsorb toxic materials such as pesticides or heavy metals from air or water; the fate of these toxic burdens depends largely on the size and density of the core particle, which determines their rate of sedimentation, ingestion, lung penetration, and so forth.

These particles can generally be considered as two-phase particles, although the core itself is in some cases (as in cells) rather heterogeneous. Characterization of these particles is a daunting task: The composition of each phase must be known and the mass or volume of each phase must be obtained. In addition, for most real-world samples with broad distributions, information on the nature of the distributions must be obtained so that the role of the subpopulations can be determined. Most analytical techniques are ill equipped to unravel the physical and chemical complexities of two-phase par-

ticles and are particularly unable to provide valid distributions of properties. FFF, used alone or in combination with other techniques, has some unique abilities in this area.

Beckett and co-workers have obtained detailed size distributions of numerous environmental colloids by FFF (25). By feeding fractions into an ICP mass spectrometer, they observed systematic shifts in element ratios with increasing particle diameter, an observation with significant geochemical implications (3, 26). Among other findings, they have obtained evidence that the iron content of colloids in river water is concentrated at the surface of the suspended particles, thus providing clues to particle genesis.

Another important secondary measurement capability of sedimentation FFF is measurement of adsorbed mass, as illustrated in Figure 5, which shows the elution diagram of three different peaks acquired for 0.2- $\mu\text{m}$  polystyrene microspheres (27). The retention shift between



**Figure 5. Sedimentation FFF of 0.2- $\mu\text{m}$  latex beads.**

The peak corresponding to beads without an adsorbed protein coat precedes those corresponding to protein-coated beads. (Adapted from Reference 26.)

peaks is due to the adsorption of minuscule amounts ( $\sim 10^{-16}$  g) of  $\gamma$ -globulin on each latex particle shown in the two right-hand peaks. These small adsorbed masses are sufficient to significantly increase the retention time, and the increment in retention times gives a direct measure of adsorbed mass using the basic equations described earlier. (Our more recent studies of IgG adsorption show that only six IgG molecules yield a perceptible shift in retention time [28].) By making measurements at different solution concentrations, protein adsorption isotherms can be obtained (27, 28). The measurement of protein adsorption is important in the design of immunodiagnostic assays and the evaluation of biomedical implants. Such measurements are usually made by indirect means (the solution depletion method) that are both laborious and error-prone. Strategies for using sedimentation FFF to probe the structures and content of a variety of other colloids with outer layers or shells have been described (1, 21, 29).

**Bulk and surface composition.** FFF is generally considered to be a family of physical techniques that respond to physical rather than chemical properties. Nonetheless, some primary properties measurable by FFF are sensitive to chemical composition. In such cases, FFF can be used to probe compositional variations in subpopulations of macromolecules and colloids. However, such secondary compositional analysis by FFF has received little attention. (A more elaborate approach is to couple FFF to composition-sensitive instruments, such as an ICP mass spectrometer, to provide a matrix of physical and chemical properties.)

Even the primary particle property, density, is fixed by composition. Although density alone does not specify composition, it can be used to distinguish among suspected compositional variants or to fix the ratio of two known components. For example, the drug load in a liposome can in theory be determined by sedimentation FFF density measurements.

Thermal FFF is also a promising tool for compositional studies, although the fundamental basis of compositional effects is not understood. It has long been known that retention is sensitive to polymer composition, an observation that has

spurred efforts to determine the compositional distribution of copolymers by thermal FFF (30).

Thermal FFF is also sensitive to the composition of colloidal particles (31, 32). Surface composition appears to play a major role. For example, silanized silica colloids are retained  $\sim 60\%$  longer in an aqueous suspension than are unmodified silica colloids. In a similar vein, surface charge, and thus electrical FFF retention, depends on surface composition and constitutes a sensitive probe of compositional shifts (33).

### Still ahead

The future of FFF measurement is bright because so many primary and secondary properties of macromolecules and colloids can be obtained, some of which are inaccessible by other techniques. However, the field is still in its infancy, with most applications still ahead. We anticipate further development of measurement strategies and thus an expanded base of measurement capabilities in the next few years. Particularly vigorous development is expected in finding and exploiting new combinations of FFF and downstream measurement techniques with increasingly powerful capabilities for probing the structure and composition of complex real-world colloidal and macromolecular materials.

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### References

- Giddings, J. C. *Science* **1993**, *260*, 1456–65.
- Giddings, J. C.; Caldwell, K. D. In *Physical Methods of Chemistry*; Rossiter, B. W.; Hamilton, J. F., Eds.; John Wiley and Sons: New York, 1989; Vol. 3B, pp. 867–938.
- Taylor, H. E.; Garbarino, J. R.; Murphy, D. M.; Beckett, R. *Anal. Chem.* **1992**, *64*, 2036–41.
- Caldwell, K. D.; Li, J. *J. Colloid Interface Sci.* **1989**, *132*, 256–68.
- Barman, B. N.; Giddings, J. C. *Langmuir* **1992**, *8*, 51–58.
- Giddings, J. C.; Caldwell, K. D.; Jones, H. K. In *Particle Size Distribution: Assessment and Characterization*; Provder, T., Ed.; ACS Symposium Series 332, American Chemical Society: Washington, DC, 1987; Chapter 15.
- Yau, W. W.; Kirkland, J. J.; Bly, D. D. *Modern Size Exclusion Liquid Chromatography*; John Wiley and Sons: New York, 1979.
- Giddings, J. C. *Unified Separation Science*; John Wiley and Sons: New York, 1991.
- Lee, T. T.; Dadoo, R.; Zare, R. N. *Anal. Chem.* **1994**, *66*, 2694–2700.
- Giddings, J. C.; Yang, F.J.F.; Myers, M. N. *Sep. Sci.* **1975**, *10*, 133–49.
- Giddings, J. C.; Karaiskakis, G.; Caldwell, K. D.; Myers, M. N. *J. Colloid Interface Sci.* **1983**, *92*, 66–80.
- Giddings, J. C. *Chem. Eng. News* **1988**, *66*(41), 34–45.
- Giddings, J. C.; Moon, M. H. *Anal. Chem.* **1991**, *63*, 2869–77.
- Williams, P. S.; Lee, S.; Giddings, J. C. *Chem. Eng. Commun.* **1994**, *130*, 143–66.
- Liu, M-K.; Giddings, J. C. *Macromolecules* **1993**, *26*, 3576–88.
- Liu, M-K.; Li, P.; Giddings, J. C. *Protein Sci.* **1993**, *2*, 1520–31.
- Giddings, J. C.; Ho, J. *Langmuir* **1995**, *11*, 2399–2404.
- Giddings, J. C. *Anal. Chem.* **1994**, *66*, 2783–87.
- Caldwell, K. D.; Gao, Y-S. *Anal. Chem.* **1993**, *65*, 1764–72.
- de Gennes, P-G. *Scaling Concepts in Polymer Physics*; Cornell University Press: Ithaca, NY, 1979.
- Moon, M. H.; Giddings, J. C. *J. Pharm. Biomed. Anal.* **1993**, *11*, 911–20.
- Everett, D. H. *Basic Principles of Colloid Science*; The Royal Society of London: London, 1988.
- Hiemenz, P. C. *Principles of Colloid and Surface Chemistry*, 2nd ed.; Marcel Dekker: New York, 1986.
- Giddings, J. C.; Barman, B. N.; Li, H. *J. Colloid Interface Sci.* **1989**, *132*, 554–65.
- Beckett, R.; Hart, B. T. In *Environmental Particles*; Buffel, J.; van Leeuwen, H. P., Eds.; CRC Press: Boca Raton, FL, 1993; Vol. 2, pp. 165–205.
- Murphy, D. M.; Garbarino, J. R.; Taylor, H. W.; Hart, B. T.; Beckett, R. *J. Chromatogr.* **1993**, *642*, 459–67.
- Beckett, R.; Ho, J.; Jiang, Y.; Giddings, J. C. *Langmuir* **1991**, *7*, 2040–47.
- Yong, Y.; Giddings, J. C.; Beckett, R. In *Proteins at Interfaces 1994*; Horbett, T. A., Ed.; ACS Symposium Series; American Chemical Society: Washington, DC, in press.
- Langwost, B.; Caldwell, K. D. *Chromatographia* **1992**, *34*, 317–24.
- Schimpf, M. E.; Giddings, J. C. *J. Polym. Sci. Part B: Polym. Phys.* **1990**, *28*, 2673–80.
- Shiundu, P. M.; Liu, G.; Giddings, J. C. *Anal. Chem.* **1995**, *67*, 2705–13.
- Shiundu, P. M.; Giddings, J. C. *J. Chromatogr.*, in press.
- Schimpf, M. E.; Caldwell, K. D. *Am. Lab.* **1995**, *30*, 1435–53.

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