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COBALT ORES: FLOTATION

See **III / NICKEL AND COBALT ORES: FLOTATION**

COLLOIDS: FIELD FLOW FRACTIONATION

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Introduction

When dealing with colloidal materials, the parameters of size and size distributions are needed, because many physicochemical processes, like aggregation and deposition on solid surfaces, are influenced by the size and size distributions of these materials.

For the analysis of submicron or supramicron colloidal particles, a sedimentation process, either under gravity or with centrifugal force, is necessary to sort different diameter particles into classes, and then to obtain an average size and a size distribution. Among the successful techniques used for this purpose, are electrophoretic mobility and the methods of size exclusion chromatography, hydrodynamic chromatography and field-flow fractionation (FFF).

FFF is a family of separation methods introduced by Giddings in 1966, but several groups all around the world have contributed significantly to the development. The various subtechniques of FFF are best suited to the separation and characterization of colloidal materials and macromolecules, including biological components ranging in size from proteins to living cells, environmental colloidal particles, as well as industrial polymers and colloids, powders, latexes and emulsions.

Principles of FFF

FFF is a flow elution method with retention achieved by using applied fields to drive the solutes into quiet flow regions. The applied fields are able to achieve the task of separation more gently and with more accurate control than the two-phase distribution forces used in chromatography.

Just as its name suggests, fractionation in FFF is the combined effect of an applied cross-field and the laminar parabolic flow profile of the carrier mobile phase. The separation process in FFF is carried out in a thin, unobstructed, ribbon-like channel with a rectangular cross-section and elongated extremities connected to capillary tubes allowing the continuous flow of the carrier liquid (**Figure 1**). Flow velocity is highest at the middle of the channel and lowest near the two channel walls. As the carrier stream moves through the channel, an external force field or gradient is applied perpendicularly to the flow, which pushes the components of the sample into the slower moving streams near the outer wall, so that they form a thin, steady-state layer against that wall.

The mean thickness of the layer is different for each distinct chemical or particulate species, and depends on the strength of the interaction between the field and the species and on the diffusion coefficient corresponding to that species. Larger colloidal species and macromolecules are usually forced more vigorously toward the wall than are smaller ones. This is the basis for selective retention in normal FFF, which produces in general an elution spectrum in which small particles are eluted first and large particles last, until steric effects dominate. At this transition point a foldback in elution order occurs, and the larger particles elute from the system before the smaller ones – a trend opposite to that observed in normal FFF. Steric FFF (StFFF) predominates when the particle radius exceeds the mean layer thickness of the solute, such that the protrusion of the particle out of the flow system is determined by the particle's size rather than by its Brownian motion.

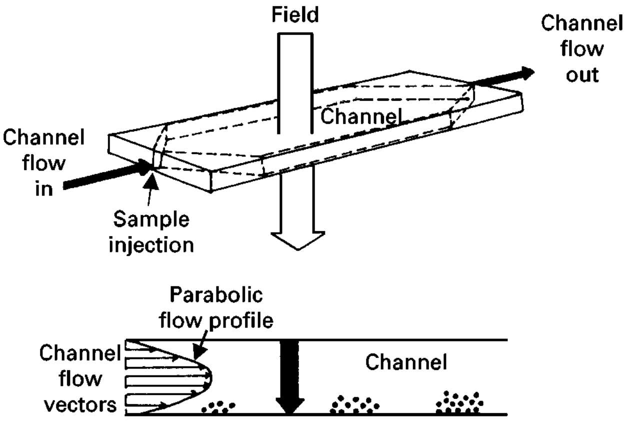

Figure 1 Schematic representation of the FFF channel with the parabolic flow profile in detail. Reproduced with permission from Giddings JC (1979) Field-flow fractionation of polymers: one-phase chromatography. *Pure and Applied Chemistry* 51: 1459.

Sub-techniques of FFF

Among the various fields and gradients used to drive species to the channel wall, four specific kinds have been most developed, yielding a different subtechnique of FFF. Among those used are centrifugal or gravitational forces (sedimentation FFF = SdFFF), thermal gradients (thermal FFF = ThFFF), cross-flow (flow FFF = FlFFF) and electrical fields (electrical FFF = ElFFF). All these subtechniques can be worked under the normal mode of operation, in which the concentration distribution along the axis which is perpendicular to the carrier flow is exponential as a consequence of the balance between field-induced displacements and diffusion, or under the steric mode of operation in which the elution order is inverted relative to that observed for normal FFF. They can also be used in the hyperlayer and potential barrier modes of operation, which will be described in detail later.

Retention Theory

The peak retention in FFF can be expressed by the retention ratio, R, defined as the ratio of the void volume, V_o, to the component retention volume, V_R:

$$R = \frac{V_o}{V_R} \qquad [1]$$

The retention ratio R in normal FFF is related to the dimensionless retention parameter λ ($\lambda = l/w$, where l is the mean layer thickness and w is the channel thickness) by the equation:

$$R = 6\lambda[\coth(1/2\lambda) - 2\lambda] \approx 6\lambda \qquad [2]$$

The last approximation is valid for highly retained components. Eqn [2] is general for all FFF subtechniques, but as the parameter λ varies with the applied external field or gradient, the experimental parameter R is related to different physicochemical quantities of the sample under study, thus leading to the sample's separation depending on its interactions with the external field:

$$R = \frac{6kN_A T\rho_s}{MG\Delta\rho} = \frac{36kT}{d^3\pi wG\Delta\rho} \quad \text{(SdFFF)} \qquad [3]$$

$$R = \frac{6T}{aw(\mathrm{d}T/\mathrm{d}x)} = \frac{6D}{D_\mathrm{T}w(\mathrm{d}T/\mathrm{d}x)} \qquad \text{(ThFFF)} \quad [4]$$

$$R = \frac{6DV_\mathrm{o}}{\dot{V}_\mathrm{c}w^2} \qquad \text{(FlFFF)} \quad [5]$$

$$R = \frac{6D}{\mu E w} \qquad \text{(ElFFF)} \quad [6]$$

$$R = \frac{3\gamma d}{w} \qquad \text{(StFFF)} \quad [7]$$

where M is the solute molecular weight, d is the Stokes diameter, $\Delta\rho = |\rho_\mathrm{s} - \rho|$ is the density difference between the particle (ρ_s) and the carrier liquid (ρ), G is the field strength expressed in acceleration, T is the absolute temperature, k the Boltzmann's constant, N_A Avogadro's number, D is the solute–solvent diffusion coefficient, D_T the coefficient of thermal diffusion, a the dimensionless thermal diffusion factor, $\mathrm{d}T/\mathrm{d}x$ is the temperature gradient applied at right angles to flow, $\dot{V}_\mathrm{c}$ is the volumetric cross-flow rate, μ is the particle's electrophoretic mobility, E is the electrical field strength, and γ is a dimensionless factor that accounts for the drag-induced reduction in velocity, and for the increase in velocity due to the activity of lift forces.

The above equations show that the solute parameters controlling retention, and thus characterized by retention, are M, d and ρ_s in SdFFF, D_T and a in ThFFF, D and d in FlFFF, μ and D in ElFFF, and d, γ in StFFF. As in all FFF techniques, the retention ratio is related to the particle size and the fractionation is based on particle size differences or on the D and D_T coefficients.

We note that all R values in the normal FFF mode are inversely proportional to the strength of the field or gradient employed: G, $\mathrm{d}T/\mathrm{d}x$, $\dot{V}_\mathrm{c}$ and E – therefore making possible the manipulation of the retention ratios by changes in field strength. This makes FFF applicable to many different sample types over a wide particle size distribution range. A wide variety of components can be handled within a run by applying field programming methodologies in which the field strength is gradually reduced during the separation.

In the present work, we focus on sedimentation FFF (centrifugal and gravitational) and flow FFF, which are the most usable and accurate for the separation and characterization of colloidal materials.

Instrumentation

Sedimentation FFF

The apparatus of SdFFF consists of a channel with highly polished walls and rectangular cross-section which is fitted to the inside of a centrifuge basket. Solvent is pumped into and out of the system with the aid of special low volume rotor seal and subsequently to a UV detector. The associated parts of the system (e.g. pumps, detectors, recorders, sample injectors) are readily available liquid chromatography components. At present, the SdFFF rotor can be operated at a maximum speed of 32 000 rpm, which corresponds to an applied field of approximately 85 000 **g** for the common rotors used. With such a rotor, colloidal particles as small as 0.005 μm can be retained and analysed, when their density is between 1.5 and 3.0 g cm^{-3}. The upper limit of particle size is reached when the mean layer thickness becomes equal to or less than the particle radius, and steric factors determine the retention ratio.

In gravitational FFF (GrFFF) the earth's gravitational field is used and the apparatus consists of a channel formed between two glass plates separated by a Mylar spacer.

Flow FFF

In flow FFF the external field is simply the flow of a second solute stream perpendicular to the carrier stream. The walls of the FFF channel are constructed of semipermeable membranes, so as to accommodate the cross-flow and, at the same time, to retain solute particles in the channel. The semipermeable membrane material is the limiting factor in the performance of the flow FFF system.

Applications

SdFFF and FlFFF can be applied to the separation and characterization of spherical or irregular, monodisperse or polydisperse colloidal particles. The particle size analysis of colloids can be carried out successfully by working in the four modes of FFF.

Normal SdFFF

Normal SdFFF has been applied to many systems involving polymers, biological macromolecules (such as DNAs, virus particles and protein aggregates), subcellular particles, emulsions and a great variety of natural and industrial colloids. An example of SdFFF is shown in **Figure 2**, which illustrates the separation of colloidal polystyrene latex microspheres. No other method can yield comparable resolution for submic-

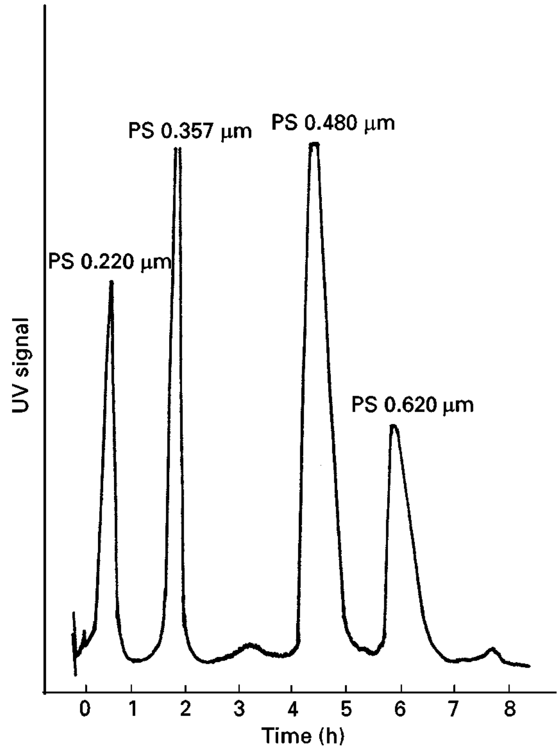

Figure 2 Separation by SdFFF of four polystyrene (PS) latex beads with the diameters indicated in the scheme. The experimental conditions were as follows: field strength 193.7 **g**; flow rate 12 cm^3 h^{-1}; void volume of the channel 2.0 cm^3. Reproduced from Giddings JC, Myers MN, Caldwell KD and Fisher SR (1980) Analysis of biological macromolecules and particles by field-flow fractionation. *Methods of Biochemical Analysis* 26: 79, with permission from Wiley and Sons, Inc.

ron particles. One of the advantages of SdFFF in characterizing colloids is the fact that they are separated into sharp fractions and that certain properties of each fraction can be deduced by the measurement of the fraction's retention time. Accurate characterization is possible without calibration because of the simplicity and theoretical tractability of the SdFFF system (cf. eqn [3]), when interactions between the particles and the channel wall are absent. Particle parameters subject to characterization include mass, density, polydispersity, diffusivity and various particle dimensions (number and weight average diameters, differential and cumulative size distributions).

A series of papers relating to colloid characterization by SdFFF has been published by Giddings. The first paper showed that relatively monodisperse colloidal populations can be characterized with respect to particle mass, diameter, density and polydispersity. Subsequent papers described methods for obtaining particle size distributions for polydisperse colloids and emulsions, and the separation and characterization of narrow populations or subsets within a polydisperse population of colloidal particles.

A wide variety of inorganic colloids of industrial importance, including ceramics, carbon black, quartz, clay silica, alumina, titania, haematite and hydroxyapatite, have been separated and characterized by SdFFF with high resolution in less than 15 min, under optimum conditions. SdFFF also permits analytical separations of submilligram quantities, and the separated components are easily collected for subsequent work. SdFFF can be used on a larger scale to separate quantities of the order of a gram of purified components. Fractionation and characterization by SdFFF of the submicron colloidal particles contained in natural waters have also been carried out. With the aid of an on-channel concentration procedure, SdFFF has been shown to be applicable to an area of analysis where the particle system in natural water is complex and the particles are present in much lower concentrations than in samples which can be analysed without the preconcentration procedure. Although particle diameter can be obtained by SdFFF only if the particle density is known, this apparent weakness can be converted into a major strength. By changing the density of the suspending medium, the difference between it and the density of the solute particles can be changed by known increments, and it becomes simple to split up the d parameter and $\Delta\rho$ terms of eqn [3]. Therefore, both particle size and density can be found by SdFFF.

Particle size distribution analyses have been carried out on a wide variety of suspended organic and inorganic particulates (such as polychloroprene and polymethyl methacrylate lattices, water-based titania and carbon black dispersions, phthalocyanine blue dyes) using the methodology of time-delayed exponential force-field sedimentation field-flow fractionation (TDE-SdFFF). Particle retention time in TDE-SdFFF correlates simply and accurately with the logarithm of particle size (diameter or mass). Relative to constant-field SdFFF, the TDE-SdFFF method provides the advantages of decreased analysis time and improved detection sensitivity, while maintaining adequate resolution for accurate differential and cumulative particle size distributions.

SdFFF has been also used for the kinetic study of aggregation of various inorganic colloids, including hydroxyapatite and sulfide samples. From the variation of the number average diameter obtained by SdFFF with time, rate constants for the bimolecular process of aggregation and stability factors of the colloids have been determined.

Normal FlFFF

FlFFF has been applied to a variety of species including silica gel, latex and virus particles, as well as proteins, protein clusters and water-soluble polymers. FlFFF has also been applied to humic materials, pushing the lower molecular weight limit to a few hundred. An asymmetric version of FlFFF has been introduced in which only one wall, the accumulation wall, is permeable to the liquid, whereas the other wall is solid. Thus, the crossflow is generated by the fraction of the inlet flow that exists through the membrane. With this asymmetric system, various biological macromolecules and water-soluble polymers have been successfully analysed.

Steric FFF

StFFF represents the upper limit of the field strength applied. Although any effective field may be applied to the StFFF mode, the gravitational field represents the most practical means of utilizing the principle of StFFF to the analysis of particles in the diameter range 1–100 μm. The first experimental evidence for the applicability of StFFF was presented in 1978 by Giddings for the fractionation of glass beads having diameters 10–32 μm. Later the technique was applied to the separation of a mixture of spherical silica beads in the diameter range 5–14 μm, to the separation of a number of large particle samples, including chromatographic support materials and fine-ground coals, as well as to useful applications in biochemistry and biology (yeast cells, whole blood).

Recently, the StFFF technique has been applied to particle size analysis (number and weight average particle diameters, as well as differential and cumulative particle size distributions) of polydisperse supramicron particles of strengite ($FePO_4 \cdot 2H_2O$ with number average diameter of 3.5 μm), which is of great significance in natural waters, and mixed sulfides ($Cu_xZn_{(1-x)}S$, $0 < x < 1$, with number average diameter in the range 5.7–7.6 μm), which are of paramount importance in industrial chemistry. The particle size distributions, which correspond to the longest axis of the needle-shaped $FePO_4$-$2H_2O$ particles, found under various experimental conditions by StFFF, are in good agreement with those obtained by transmission electron microscopy (TEM). This confirms the feasibility of the StFFF technique for characterizing polydisperse, irregular, inorganic colloids, and verifies the general features of the theory.

From the variation of the number and weight average diameter of the $Cu_xZn_{(1-x)}S$ particles determined by GrFFF with various parameters, such as solution pH and ionic strength, kind of detergent and time, in combination with microelectrophoresis measurements, useful conclusions about the stability and consequently the aggregation and deposition phenomena of these sulfides can be deduced.

In the normal mode of SdFFF operation the relationship between V_R and d can be established using eqn [3], without empirical calibration. In the steric mode of SdFFF (Sd/StFFF), calibration must be established empirically, as the steric correction factor in eqn [7] is unknown, increasing with the flow velocity and decreasing with the increase of field strength. As retention is based on particle density as well as size, a purely size-based calibration is not generally valid. By examining the balance between driving and lift forces, it is observed that an equal retention ratio is obtained for equal size particles subject to equal driving forces independently of the particle density. Thus, by adjusting the field strength G to compensate exactly for the density difference $\Delta\rho$, so that the product $G\Delta\rho$ in eqn [3] is the same for the sample and the standard polystyrene latexes, linear calibration plots of log(retention time) versus log(diameter) for the latexes can be used to characterize the sample under study. This calibration procedure for Sd/StFFF has been successfully applied to the rapid size separation and measurement of particle size distribution of various starch granules.

Hyperlayer FFF (HyFFF)

The sedimentation hyperlayer field-flow fractionation (SdHyFFF) technique differs from conventional FFF methods in that the solute zone is focused into a thin layer by the opposing forces of centrifugal acceleration and buoyancy in a density gradient, which is formed while the carrier is flowing through the channel. So an equilibrium distribution is established by the time the fluid reaches the downstream injection size. Solutes with low molecular weights require impractically high field strengths to form substantial gradients, whereas particulate density modifiers establish the gradient both rapidly and effectively. Ideally each particle type in SdHyFFF is focused into a very thin layer at its own characteristic elevation. Here it occupies a thin fluid layer flowing at a fixed velocity. The difference of SdHyFFF from the normal SdFFF is that it is the only FFF methodology for which each distinct species is narrowly confined to its own unique velocity state.

The applications of SdHyFFF include the fractionation of polystyrene latex and biological particles (human and animal cells). With the introduction of denser particles its effective density range has been extended to silica, thus making the technique applicable to environmental particles. Another application of SdHyFFF technique is the fractionation of coal and limestone particles.

In general, the SdHyFFF method is applicable to larger particles than SdFFF, where (wall-induced) steric effects become important for particle sizes around 1 μm. The focusing of sample zones at locations removed from the wall in SdHyFFF has the advantage of eliminating interface-related nonidealities, such as sample adhesion and zone broadening caused by surface roughness.

Important developments include the so-called hyperlayer flow FFF, which has extended the applications to particles larger than 1 μm in diameter. Also, the introduction of various programming procedures has been of importance. A number of reports of the combined use of FlFFF and multi-angle laser light scattering (MALLS) instruments have also been published, illustrating the usefulness of this hyphenated technique in characterizing colloidal particles.

Potential Barrier FFF (PBFFF)

Potential barrier field-flow fractionation (PBFFF), developed by Karaiskakis, is a combination of potential barrier chromatography and FFF. Potential barrier chromatography can be applied to separate particles based on differences in size or in any of the physicochemical parameters involved in the potential energy of interaction between the particles and the packing of the chromatographic column. The particle size analysis of colloidal materials by PBFFF is based either on particle size differences or on Hamaker constant, surface potential and Debye–Huckel reciprocal differences. The method, which is based on the existence of a surmountable potential barrier between the colloidal particles and the FFF channel wall, is classified as an FFF method rather than a chromatographic method, because the selective interaction is experienced in one phase. Thus, by combining potential barrier chromatography with normal or steric FFF one could separate according to two mechanisms, one governed by the depth of the potential energy well for the different particles and the other determined by the interactive force between the particles and the external field. In its simplest form the

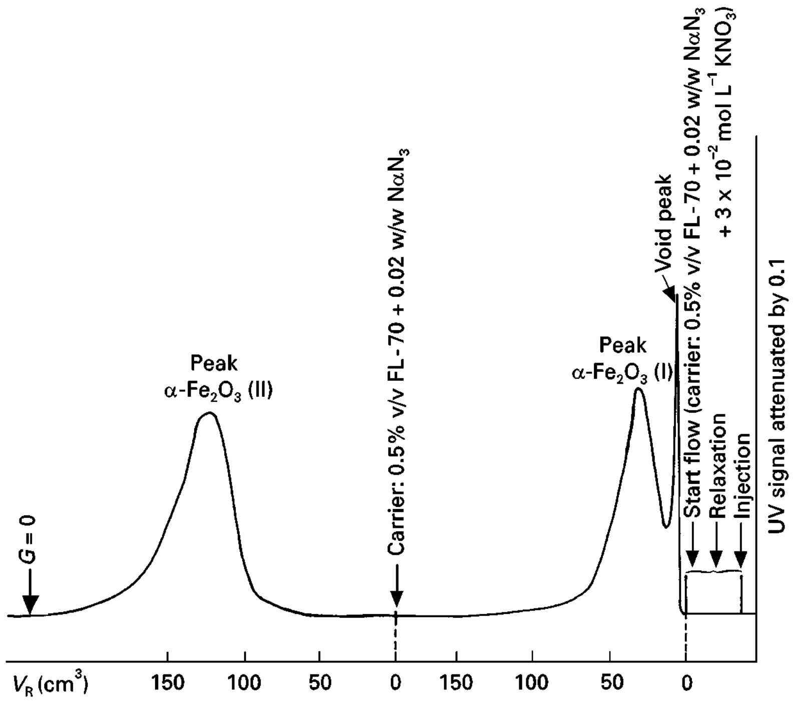

Figure 3 Fractionation of haematite-I (α-Fe_2O_3(I) with nominal diameter 0.148 μm) and haematite-II (α-Fe_2O_3(II) with nominal diameter 0.248 μm) colloidal particles by the potential barrier SdFFF technique. The experimental conditions, expect for those given in the scheme, were as follows: field strength 15.5 **g**; flow rate 150 $cm^3\,h^{-1}$; void volume of the channel 2.06 cm^3. Reproduced from Koliadima A and Karaiskakis G (1990) Potential-barrier field-flow fractionation, a versatile new separation method. *Journal of Chromatography* 517: 345, with permission from Elsevier Science.

technique consists of changing the ionic strength of the carrier solution from a high value, where only one of the colloidal materials of the binary mixture to be separated totally adheres at the beginning of the FFF channel wall, to a lower value, where all the adhered particles are released.

Figure 3 shows the fractionation of two haematite samples (α-Fe_2O_3(I) with nominal particle diameter 0.148 μm and α-Fe_2O_3(II) with nominal particle diameter 0.248 μm) with monodisperse spherical particles, by the PBSdFFF technique. The mixture was introduced into the channel with a carrier solution containing 0.5% v/v detergent FL-70, 0.02% w/w NaN_3 and 3×10^{-2} mol L^{-1} KNO_3. At this high electrolyte concentration all of the α-Fe_2O_3(II) particles adhered at the beginning of the SdFFF Hastelloy-C channel wall, whereas all of the α-Fe_2O_3(I) particles were eluted from the channel. The average diameter of the eluted α-Fe_2O_3(I) particles was found from eqn [3] to be 0.151 μm, in good agreement with that obtained by normal SdFFF (0.145 μm) or determined by TEM (0.148 μm). Variation of the carrier solution to one containing only 0.5% v/v detergent FL-70 and 0.02% w/w NaN_3 without any KNO_3 released all the adhered α-Fe_2O_3(II) particles and gave a particle diameter (0.244 μm) in good agreement with that found by normal SdFFF (0.237 μm) or obtained by TEM (0.248 μm).

A general methodology for the analysis of a colloidal mixture by PB SdFFF consists of injecting into the column the mixture with a carrier solution in which the ionic strength is too high to ensure total adhesion of all the components of the mixture, except for one with the lower attractive force with the channel wall. Then a programmed variation (decrease) of the ionic strength of the carrier solution is applied to release, in time, the adherent particles according to their size and/or surface characteristics. As the PBSdFFF technique is based on particle–wall interactions, its applications can be extended by using different materials, such as stainless steel, Teflon and polyimide. PBSdFFF is also a convenient and accurate method for the concentration and analysis of dilute colloidal samples. This makes PBSdFFF a highly attractive technique for the characterization of samples where even particles of the same size but of different chemical composition are present in low concentration.

Future Developments

From the detailed description of the FFF techniques presented above, it is concluded that the most usable FFF methods for particle size analysis of colloids are SdFFF and FlFFF. Looking to the future, it is reasonable to expect serious efforts by the companies producing FFF equipments, to improve their resolution and expand the particle size range to lower limits of analysis. As far as FFF applications are concerned, it is believed that future efforts will be focused on the size separation and characterization of polydisperse, irregular colloidal particles, as well as on the aggregation and deposition phenomena of colloids on solid surfaces.

See also: **II/Particle Size Separation:** Field Flow fractionation: Electric Fields; Field Flow Fractionation: Thermal; Theory and Instrumentation of Field Flow Fractionation. **III/Cells and Cell Organelles: Field Flow Fractionation. Colloids: Field Flow Fractionation. Polymers:** Fixed Flow Fractionation. **Proteins:** Field Flow Fractionation.

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