plants. The long-term goal of the process is to replace packed towers in conventional absorber–stripper operations. Practical problems related to membrane fouling and lifetime are the principal limitations.

The Future

Since the 1970s there has been a period of very rapid growth for the membrane separation industry. Total sales for all membrane applications have grown approximately 400-fold to the US$3–4 $\times 10^9$ per year level. In the areas of microfiltration, ultrafiltration, reverse osmosis, electrodialysis and dialysis, the technology is relatively mature. Significant growth is still occurring, however, as membranes continue to displace more conventional separation techniques. The most rapidly expanding area is gas separation, which has grown to a US$150 $\times 10^6$ per year business in just a few years. Gas separation is poised to grow a further two- or three-fold as the technology is used more widely in the refinery, petrochemical and natural gas processing areas. If the development of ceramic oxygen-permeable membranes for syngas membrane reactors is successful, a membrane process that could change the basis of the chemical industry would then be available.

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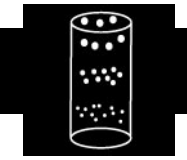
PARTICLE SIZE SEPARATIONS



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Historical Development

In 1556, an extraordinary book entitled *De Re Metallica*, *Libri XII* appeared in Basel. The author was a German physician, naturalist and mineralogist, calling himself Georgius Agricola (originally called Georg Bauer), living in Jáchymov, Bohemia, from 1494 to 1555. Agricola described, in a fascinating manner, the contemporary advances in metals and minerals recovery and gave us a very detailed report on the sophisticated technologies of his epoch. This late medieval period saw a true expansion of science and technology in Europe. Winston Churchill once said: '… from this date, 1492, a new era in the history of mankind takes its beginning'. As many metal recovery processes used at that time were based on various separations of particulate matter and *De Re Metallica*, *Libri XII* seems to be the first printed review of separation technologies, it is fitting to acknowledge Agricola's publication priority in this field and to consider his book as the beginning of a modern scientific approach to particle size separations.

The reproduction of a rendering in **Figure 1** taken from Agricola's book shows a surprisingly sophisticated device for gold (and other metals) recovery by 'panning' or 'sluicing' which used gravity and

[1] This article does not deal with the important particle separation techniques of filtration, flotation and the use of membranes which are dealt with elsewhere in the Encyclopedia.

Figure 1 Mediaeval device for the recovery of gold particles and minerals from sand, clay, and soil blends by combining the sedimentation and quasi-horizontal stream of water, accompanied by vigorous manual stirring of the mud cake. (Bottom) The author of the book *De Re Metallica*, *Libri XII*, Georgius Agricola.

a stream of running water to separate gold particles from other solid material (soil, clay, sand, etc.). Astonishingly, this technology dates back to at least 4000 to 5000 BC.

Original scientific discoveries, outstanding inventions and innovations in technology representing the important achievements at a given moment reflect continuity of imagination throughout the long history of civilization. When looking for the background and genesis of modern and powerful separation methodologies and technologies, very often natural analogies can be found at a macroscopic level. An image of a river meandering through the countryside and removing soil, clay, sand, and stones from a river bank, carrying them off in the stream, and depositing them later at other places, is one such example. On the other hand, although ancient technologies can have essentially the same goal (separation), in a manner similar to that in which 'cat's cradle' is equivalent to a sophisticated electronic computer game, the intellectual progress is evident.

Dry and wet sieving, sedimentation, and filtration are probably the most ancient, intelligently applied, separation processes on which the foundations of modern separation science stand. These processes were originally exploited for the separations of disintegrated matter whose average 'particle' size was somewhere between millimetre and centimetre fractions, sometimes even bigger. Slowly, the need to separate smaller and smaller particle size material became apparent. The old-fashioned but transformed methods still afforded positive answers to questions which appeared in relation to the new separation problems. However, these transformations gave rise to newer methods which, together with the discovery and invention of completely new principles, symbolize the state of the art of particle separation.

Particles, Sizes, and Methods

In order to make clear what this article deals with, the useful and necessary terms, limits and conditions must be defined. *Particles*, within the frames of this text, is an ensemble of single subjects of disintegrated matter which is dispersed in a continuum fluid or *in vacuo*. One particle, regardless of its size, is usually not identical with one molecule but with a large number of molecules aggregated by physical forces. In the case of polymeric matter, however, one macromolecule can be identified with one particle, under certain conditions. The second important attribute which defines one particle is that, physically, it represents a subject delimited in three-dimensional space by a phase discontinuity. The particles, representing one discontinuous phase which can be solid or liquid, are dispersed in a second continuous phase which is gaseous or liquid.

As concerns the *sizes* of the particles, a strict definition is less easy, because the effective dimension(s) (independently of the physical shape of each individual particle) can vary as a function of the

chemical character of the surrounding dispersing fluid but also of the imposed physical conditions: obvious ones, such as, e.g., the temperature, and less obvious as, e.g., the electric charge, etc. Moreover, it has to be taken into account that the results of the measurements of the particle size can strongly depend on the method of its determination. As a result, the questions are not only what the size that we obtain from a particular measuring method means and whether the result corresponds to a true size, but also what kind of effective size we measure by applying any particular method. Not only one but many effective sizes obtained by different measuring methods can correspond to the physical reality (they all can be 'true'). This is due to the fact that the measured data can contain various information on the particle-dispersing fluid and particle–particle interactions, on the size fluctuations in time, on the transport behaviour of the particles in the dispersing fluid, etc. Although all these phenomena can complicate the determination of a definite particle size, they provide much useful information on the whole dispersed particulate system. Having in mind these complications, we can define the range of particle sizes of practical interest as lying within the range from a diameter of few nanometres to thousands of micrometres.

The definition and limitation of the particles and the particle size ranges, as outlined, determine the relevant separation *methods*. Those methods can be considered relevant that are directly related to the separation according to differences in particle size or concerned indirectly due to the fact that they can provide complementary information necessary to an accurate interpretation of the experimental data obtained from particle size-based separations.

Objectives and Methods

The aim of any separation, including particle size separation, is either analytical or preparative. *Analytical separations* are generally used to increase the sensitivity or selectivity of the subsequent analytical measurement, or to obtain more specific information about the analysed sample. Very often, the original sample is a complicated mixture making the analysis possible only with a prior separation step. Hence, the original multicomponent sample to be analysed must first be separated into more or less pure fractions. Whenever the samples are of particulate character and/or of biochemical or biological origin, direct analysis without preliminary separation is often impossible. An accurate analytical result can be obtained from any analytical separation method by employing an appropriate treatment and interpretation of the experimental data. Separation is usually based on the differences in extensive properties, such as the mass or size of the particles, or according to intensive properties, such as density, electrophoretic mobility, etc. If the relationship between the separation parameters and the size of the separated particles is known or can be predetermined by using an appropriate calibration procedure, the characteristics of an unknown analysed sample can be evaluated quantitatively. The particle size distributions of the analysed samples are determined conveniently from the record of a coupled detector: a fractogram. Detailed information concerning the associated properties of the separated and characterized particles and/or composition of the analysed system which can be extracted from the fractogram represents more sophisticated application of a particular separation method.

Preparative separations are aimed at obtaining a significant quantity of the separated fractions from the original sample. The fractions are subsequently used for research or technological purposes, for detailed analysis of various effective sizes, for the determination of the structure or chemical composition of the particles of a given size, etc. The practical preparative separations can range from laboratory microscale, which cannot be experimentally distinguished from analytical separations, up to industrial macroseparation units.

Analytical and preparative separations are fundamentally identical so that, consequently, we do not distinguish between them and all separation methods are described and discussed from the point of view of the principles involved by making comments on their specific applications only if the discussed technique exhibits particular characteristics predetermining it for a special analytical or preparative purpose.

The most suitable and widespread methodologies for particle size separations described below, starting from the most versatile to more specific ones, are:

- field-flow fractionation
- size-exclusion chromatography
- hydrodynamic chromatography
- centrifugation
- electrophoresis

Besides these modern techniques, some classical procedures mentioned above such as wet or dry sieving, filtration, etc., should not be forgotten.

Field-Flow Fractionation

Field-flow fractionation (FFF) is a relatively new but important and versatile method suitable for the separation and characterization of particles in the submicron and micron ranges. It has been developed over the last three decades into a complex of specific methods and techniques.

Principle of Separation

Separation in FFF is based on the action of effective physical or chemical forces across the separation channel in which the particles are transported due to the flow of a carrier liquid. The field interacts with the particles, separating and concentrating them at the appropriate positions inside the channel. The concentration gradient so formed induces an opposition diffusion flux. When equilibrium is reached, a stable concentration distribution of the particles across the channel is established. Simultaneously, a flow velocity profile is formed across the channel in the longitudinal flow of the carrier liquid. As a result, the particles are transported longitudinally at different velocities depending on the transverse positions of their zones and are thus separated. This principle is shown in **Figure 2**. The carrier liquid is pumped through the sample injector to the fractionation channel. The detector connected at the end allows the recording of the fractogram.

Separation Mechanisms

Two particular mechanisms, *polarization* and *focusing*, can govern the separation. The components of the fractionated sample can be differently compressed to the accumulation wall of the channel or focused at different levels. Polarization and focusing FFF have many common characteristics such as the experimental procedures, instrumentation, data treatment, and the range of potential applications. The separation is carried out in one liquid phase. The absence of a stationary phase of large surface area can be of fundamental importance for the fractionation of biological particles whose stability against degradation can be sensitive to interactions with the surfaces. The strength of the field can be easily controlled to manipulate the retention. Many operational variables can be programmed.

The *polarization FFF* methods are classified with regard to the character of the applied field, while the *focusing FFF* methods are classified according to the combination of various fields and gradients. Although some earlier separation methods are also based on the coupled action of field forces and hydrodynamic flow, the beginning of FFF proper can be attributed to Giddings who in 1966 described the general concept of polarization FFF. Focusing FFF was originally described in 1982.

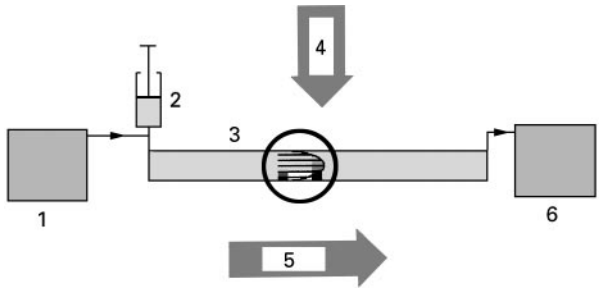

Figure 2 Schematic representation of the general principle and experimental arrangement of field-flow fractionation: (1) pump; (2) injector; (3) separation channel; (4) external field; (5) hydrodynamic flow; (6) detector.

Polarization FFF methods make use of the formation of an exponential concentration distribution of each sample component across the channel with the maximum concentration at the accumulation wall which is a consequence of constant and position-independent velocity of transversal migration of the affected species due to the field forces. This concentration distribution is combined with the velocity profile formed in the flowing liquid.

Focusing FFF methods make use of transversal migration of each sample component under the effect of driving forces that vary across the channel. The particles are focused at the levels where the intensity of the effective forces is zero and are transported longitudinally according to their positions within the established flow velocity profile. The concentration distribution within a zone of a focused sample component can be described by a nearly Gaussian distribution function.

Retention

The retention ratio R is defined as the average velocity of a retained sample component divided by the average velocity of the carrier liquid which is equal to the average velocity of an unretained sample component:

$$R = \frac{v_{\mathrm{r,ave}}}{\langle v(x) \rangle}$$

FFF is usually carried out in channels of simple geometry allowing calculation of the rigorous relationship between the retention ratio and the size of the separated particles. If this relationship is difficult to determine, a calibration can be applied. The particle size distribution (PSD) in both cases is determined from the fractogram.

Zone Dispersion

The separation process is accompanied by the zone spreading which has a tendency to disperse the concentration distribution already achieved by the separation. The conventional parameter describing the efficiency of the separation is the height equivalent to a theoretical plate H:

$$H = L\left(\frac{\sigma}{V_{\mathrm{R}}}\right)^2$$

where V_R is the retention volume and σ is the standard deviation of the elution curve. The width of the elution curve reflects several contributions: longitudinal diffusion, nonequilibrium and relaxation processes, and spreading due to the external parts of the whole separation system such as injector, detector, connecting capillaries, etc. The sum of all contributions results in a curve shown in **Figure 3** which exhibits a minimum. As the diffusion coefficients of the particles are very low, the longitudinal diffusion is practically negligible and the optimal efficiency (the minimum on the resulting curve) is situated at very low flow velocity. The instrumental and relaxation spreading can be minimized by optimizing the experimental conditions.

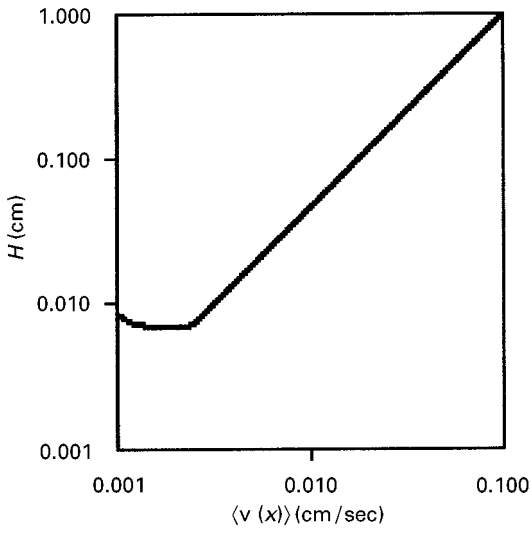

Figure 3 Dependence of the efficiency of FFF, expressed as the height equivalent to a theoretical plate H, on the average linear velocity of the carrier liquid $\langle v(x) \rangle$.

Applications of Polarization FFF

The character of the applied field determines the particular methods of polarization FFF. The most important of them are:

- sedimentation FFF
- flow FFF
- electric FFF
- thermal FFF

Sedimentation FFF is based on the action of gravitational or centrifugal forces on the suspended particles. The sedimentation velocity is proportional to the product of the effective volume and density difference between the suspended particles and the carrier liquid. The channel is placed inside a centrifuge rotor, as shown in **Figure 4**. The technique can be used for the separation, analysis and characterization of polymer latex particles, inorganic particles, emulsions, etc. The fractionation of colloidal particles in river water, diesel exhaust soot, and of the nuclear energy-related materials, are typical examples of the use of sedimentation FFF in the investigation of environmental samples. Droplets of liquid emulsions can also be separated and analysed. Biopolymers and particles of biological origin (cells) belong to the most interesting group of objects to be separated by sedimentation FFF. The performance of sedimentation FFF is superior to, or as good as, those of other separation methods. A complication in interpreting the experimental data is due to the fact that the retention is proportional to the product of particle size and density. When performing the fractionation in one carrier liquid only, the density must be assumed constant for all particles. However, it is possible to determine the size and density of the particles independently if the fractionations are performed in carrier liquids of various densities.

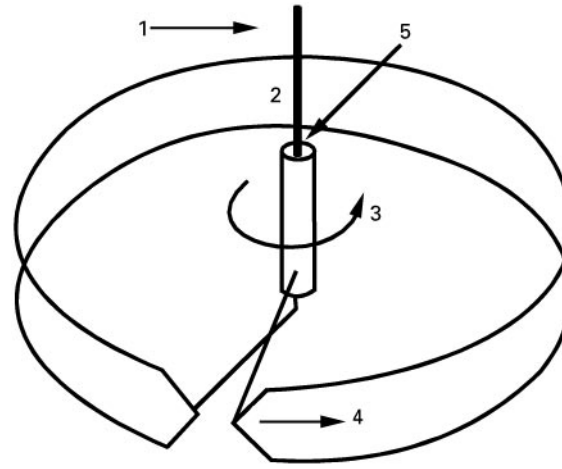

Figure 4 Design of sedimentation FFF channel: (1) flow in; (2) channel; (3) rotation; (4) flow; (5) flow out.

An example of a typical application of sedimentation FFF shown in **Figure 5** allowed detection of a bimodal PSD in a sample of a polymer latex. The order of the elution from the small to the large diameter particles corresponds to the polarization mechanism. **Figure 6** shows a rapid, high resolution sedimentation FFF of the polymer latex particles. In this case, the mechanism of steric FFF dominates, and the order of the elution is inverted.

Flow FFF is a universal method because different size particles exhibit differences in diffusion coefficients which determine the separation. The cross-flow, perpendicular to the flow of the carrier liquid along the channel, creates an external hydrodynamic field which acts on all particles uniformly. The channel, schematically demonstrated in **Figure 7**, is formed between two parallel semipermeable

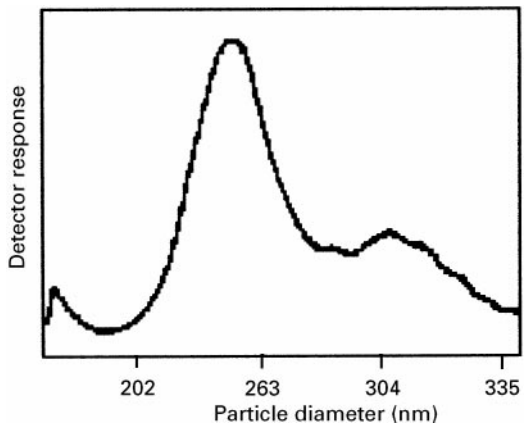

Figure 5 Fractogram of poly(glycidyl methacrylate) latex showing a bimodal character of the PSD.

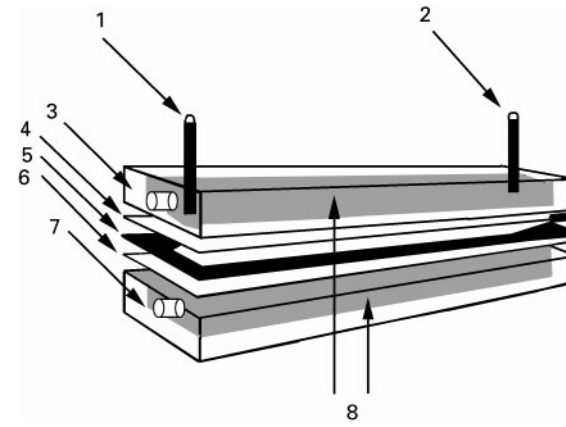

Figure 7 Design of flow FFF channel: (1) flow in; (2) flow out; (3) cross-flow input; (4) membrane; (5) spacer; (6) membrane; (7) cross-flow output; (8) porous supports.

membranes fixed on porous supports. The carrier liquid can permeate through the membranes but the separated particles cannot. Separations of various kinds of particles such as proteins, biological cells, colloidal silica, polymer latexes, etc., have been described.

Electric FFF uses an electric potential drop across the channel to generate the flux of the charged particles. The walls of the channel are formed by semipermeable membranes as in flow FFF. The particles exhibiting only small difference in electrophoretic mobilities but PSD and, consequently, important differences in diffusion coefficients, can be determined. The advantage of electric FFF compared with electrophoretic separations, e.g., with capillary electrophoresis, is that high electric field strength can be achieved at low absolute values of the electric potential due to the small distance between the walls of the channel. Electric FFF is especially suited to the separation of biological cells as well as to charged polymer latexes and other colloidal particles. The fractionation of the charged particles represents a vast application field for exploration.

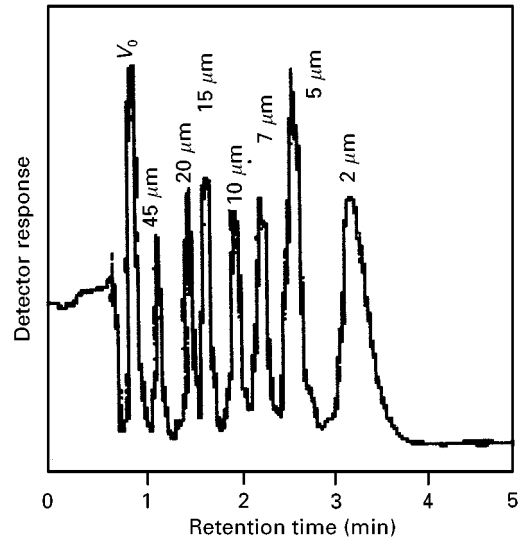

Figure 6 Fractogram of high-speed high resolution sedimentation FFF of latex beads.

Thermal FFF was the first experimentally implemented technique, introduced several years ago. Until now, it has been used mostly for the fractionation of macromolecules. Only very recently have attempts been made to apply this method to the fractionation of particles. The potential of thermal FFF justifies a description here, regardless of its recent limited use in particle separations. The temperature difference between two metallic bars, forming channel walls with highly polished surfaces and separated by a spacer in which the channel proper is cut, produces a flux in the sample components, known as the Soret effect, usually towards the cold wall. The particle sizes can be evaluated from an experimental fractogram by using an empirical calibration curve constructed with a series of samples of known sizes. This calibration can be used to determine the characteristics of an unknown sample of the same chemical composition and structure, with the same temperature gradient applied. The pressurized separation systems permit operation above the normal boiling point of the solvent used. The fractionations can be achieved in few minutes or seconds. The performance parameters favour thermal FFF over competitive methods.

Applications of Focusing FFF

Focusing FFF methods can be classified according to various combinations of the driving field forces

and gradients. The gradients proposed and exploited are:

- effective property gradient of the carrier liquid
- cross-flow velocity gradient
- lift forces
- shear stress
- gradient of the nonhomogeneous field action

Focusing can appear due to the *effective property gradient of the carrier liquid* in the direction across the channel combined with the primary or secondary transversal field. The density gradient in sedimentation–flotation focusing field-flow fractionation (SFFFFF) or the pH gradient in isoelectric focusing field-flow fractionation (IEFFFF) has already been implemented for separation of polystyrene latex particles and of biological samples. Separation by SFFFFF is carried out according to the density difference of the latex particles. An electric field can be applied to generate the density gradient in a suspension of charged silica particles. The separation by IEFFFF is carried out according to the isoelectric point differences by using the electric field to generate the pH gradient and to focus the sample components. A simple design of a channel for SFFFFF is shown in **Figure 8** and an example of the separation of two latex particles according to small density difference is demonstrated in **Figure 9**. The separation is very rapid and much less expensive when compared to isopycnic centrifugation.

The effective property gradient of the carrier liquid, e.g., the density gradient, can be preformed at the beginning of the channel and combined with the primary or secondary field forces. A step density gradient is formed in such cases but the preforming is not limited to a density gradient.

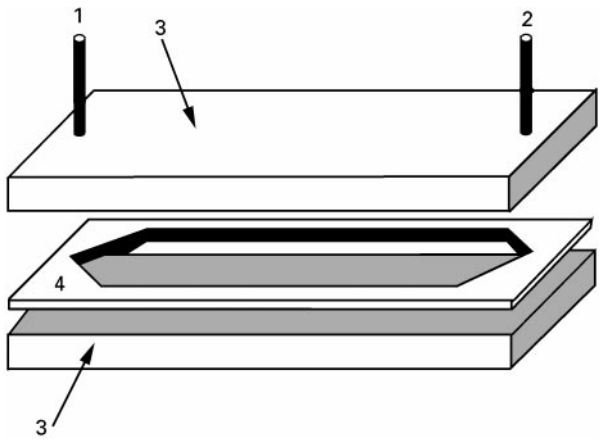

Figure 8 Schematic representation of the channel for focusing FFF in coupled electric and gravitational fields: (1) flow in; (2) flow out; (3) channel walls forming electrodes; (4) spacer.

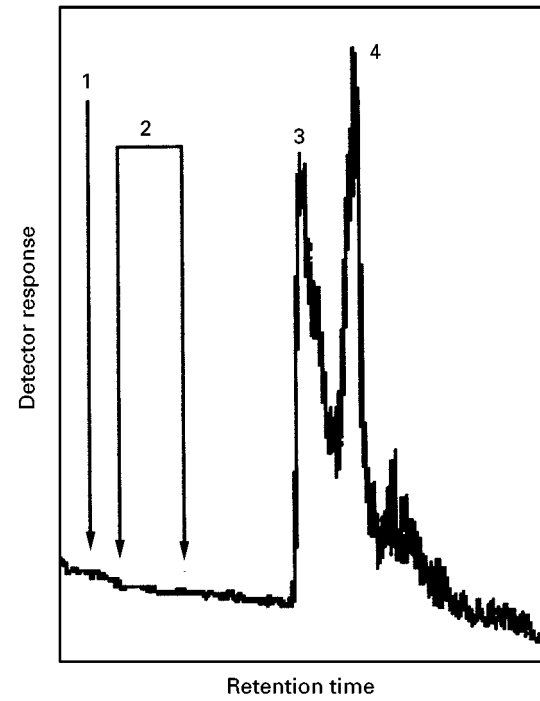

Figure 9 Fractogram of two samples of polystyrene latex particles showing a good resolution obtained by focusing FFF while no detectable resolution was achieved under static conditions: (1) injection; (2) stop-flow period; peaks corresponding to particle diameters of 9.87 μm (3) and 40.1 μm (4).

The focusing appears in the *gradient of transverse flow velocity* of the carrier liquid which opposes the action of the field. The longitudinal flow of the liquid is imposed simultaneously. This elutriation focusing field-flow fractionation (EFFFF) method has been investigated experimentally by using a trapezoidal cross-section channel to fractionate micrometre-size polystyrene latex particles but the use of the rectangular cross-section channel is possible.

The *hydrodynamic lift forces* that appear at high flow rates of the carrier liquid combined with the primary field are able to concentrate the suspended particles into the focused layers. The retention of the particles under the simultaneous effect of the primary field and lift forces generated by the high longitudinal flow rate can vary with the nature of the various applied primary field forces.

The *high shear gradient in a carrier liquid* can lead to the deformation of the soft particles. The established entropy gradient generates the driving forces that displace the particles into a low shear zone. At a position where all the driving forces are balanced, the focusing of the sample components can appear. Although this method was originally proposed by applying a temperature gradient acting as a primary field and generating the thermal diffusion flux of the macromolecules which opposes the flux due to the

entropy changes generated motion, it should be applicable to soft particles as well.

A *nonhomogeneous high-gradient magnetic field* can be used to separate various paramagnetic and diamagnetic particles of biological origin by a mechanism of focusing FFF. A concentration of paramagnetic particles near the centre of a cylindrical capillary and the focusing of diamagnetic particles in a free volume of the capillary should occur. No experimental results have yet been published.

Other gradients and a variety of the fields can be combined to produce the focusing and to apply these phenomena for PSD analysis. This review of the mechanisms used in focusing FFF should give an idea of their potential.

Size-Exclusion Chromatography

Size-exclusion chromatography (SEC) is utilized for the fractionation and analytical characterization of macromolecules but also for the separation of particles. The term gel-permeation chromatography (GPC) is used simultaneously in the literature with almost equal frequency. Other terms employed to describe this separation method are steric-exclusion liquid chromatography, steric-exclusion chromatography, gel filtration, gel-filtration chromatography, gel chromatography, gel-exclusion chromatography, and molecular-sieve chromatography. Each reflects an effort to express the basic mechanism governing the separation but the appropriate choice is more a question of individual preference.

The historical origins of SEC date from the late 1950s and early 1960s. Using cross-linked dextran gels swollen in aqueous media, Porath and Flodin separated various proteins according to their sizes. The 'soft gel' column packing used in these experiments was applicable only at low pressure and, consequently, at low flow rates resulting in very long separation times. The first successful separation of a synthetic polymer by SEC was described by Vaughan who succeeded in separating low molar mass polystyrene in benzene on a weakly cross-linked polystyrene gel. Some years later, Moore described the separation of polymers on moderately cross-linked polystyrene gel column packings.

The first rigid macroporous packing, suited also for the separation of particles, was porous silica introduced in 1966 by De Vries and co-workers. This packing was fully compatible with both aqueous and organic solvents, exhibited a very good mechanical stability, but its use was restricted by strong nonsteric exclusion interactions between the silica surface and a number of separated species. In 1974, the appearance of the packings of small porous particles with a typical diameter around 10 μm, instead of 50–100 μm particle diameter used in conventional SEC columns, resulted in an important technological improvement in SEC. The high pressure technology, the lowering of the column volume due to the use of small particle diameter packings and the high efficiency of the columns allowed the separation time to be reduced from hours to minutes. Other porous silica microparticle packings, introduced by Kirkland, Unger, and others, were resistant to the high pressure and compatible with the quasi-totality of the solvents. The undesired interactions were suppressed by organic grafting or by organic coating of the porous silica.

Principle of Separation

The separation mechanism can be explained on the basis of a specific distribution of the separated particles between the eluent outside the porous particles of the column packing (mobile phase) and the solvent filling the pores (stationary phase). This distribution is due to the steric exclusion of the separated particles from a part of the pores according to the ratio of their size to the size of the pores. The particles whose sizes are larger than the size of the largest pores cannot permeate the pores, passing only through the interstitial volume, i.e., through the void volume between the particles of the column packing, whereas very small particles may permeate all the pores. Particles of intermediate size are, to a greater or lesser extent, excluded from the pores. Hence, the elution proceeds from the largest particles to the smallest ones. This mechanism is schematically demonstrated in **Figure 10**.

The total volume of a packed chromatographic column, V_t, is given by the sum of the total volume of the pores, V_p, the volume of the matrix proper of the porous particles, V_m, and the interstitial or void volume, V_o, between the porous particles:

$$V_t = V_p + V_m + V_o$$

The retention volumes, V_R, of the separated particles lie within V_o and $V_o + V_p$. V_R of a uniform particle size fraction of the sample is defined as a volume of the eluent that passes through the column from the moment of the sample injection to the moment when the given particles leave the separation system at their maximal concentration. The retention can alternatively be expressed in time units as the retention time t_R. The particles permeating the pores are excluded from some of the pores and partially permeate the accessible pores. The retention volume of a given species can be written as:

$$V_R = V_o + K_{sec} V_p$$

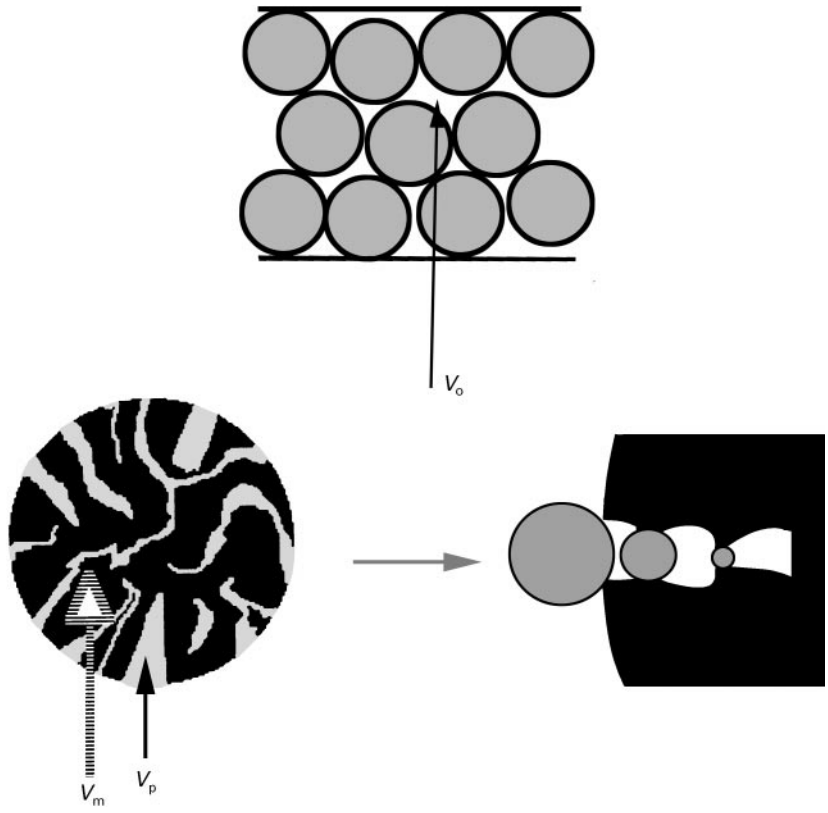

Figure 10 Schematic representation of the chromatographic column for SEC. Column with the void volume between the spherical particles of the column packing, the structure of one porous particle with the pore and matrix volumes, and the imaginary shape of one pore allowing the total permeation of smallest separated particles, partial permeation of intermediate size particles, and exclusion of largest particles.

where K_{sec} is the formal analogue of the distribution coefficient between the mobile and stationary phases.

Separation Mechanisms

Many attempts have been made to explain the mechanism of separation in SEC but steric exclusion (or size exclusion) is accepted to be the main process governing the separation. This mechanism is based on a thermodynamic equilibrium between stationary and mobile phases. As the nature of the solvent is the same in both phases, the question is to explain the dependence of the distribution coefficient K_{sec} on the size of the separated species. One of the simplest approaches uses the above-mentioned geometrical models; nevertheless, the retention volume is determined not only by the accessibility of a part of the volume of the individual pores but also by the size distribution of the entire system of pores in the column packing material. The distribution coefficient for an individual pore depends on the ratio of the pore size to the size of the separated particles and can be expressed by:

$$K_{sec} = \frac{c_p}{c_o}$$

where the concentrations c_p and c_o refer to the pores and the interstitial volume. If the pore size distribution of the column packing particles is taken into consideration, the retention volume is given by:

$$V_R = V_o + \int_R^{r_{max}} K(R, r)_{sec}\phi(r)\,dr$$

where $\phi(r)dr$ is the total volume of the pores whose radii lie within r and $r + dr$, and R is an equivalent radius of the retained particles. Hence, the retention volume of a given particulate species is determined coincidentally by the accessibility of a part of the volume of the individual pores and by the size distribution of the entire system of pores inside the column packing particles. Although different column packings exhibit almost identical dependences of V_R on separated particles size, porosimetric measurements indicate various pore size distributions. This means that the relationship between the pore size distribution and the retention volume of the separated species is not so straightforward.

An interesting model of separation by flow was proposed by Di Marzio and Guttman. The porous

structure of the SEC column packing is approximated by a system of cylindrical capillaries. The separated species move down the pores by the action of the flow but cannot get nearer to the pore wall than a distance determined by their radius. Consequently, they move at a velocity higher than the average velocity of the liquid flow due to a parabolic flow–velocity profile established in an imaginary cylindrical pore. Hence, the retention is determined by the ratio of the pore to the particle diameter. There are several factors that militate against this separation mechanism. The model assumes that the liquid can flow through the pores, which will not be true in most cases with polymeric gel particles used as column packing materials. Moreover, even in those cases when the pores are open to through flow, their diameter in comparison with the size of the interstitial voids cannot allow the flow rate to be high enough to explain the real values of the retention volumes. For the same reason, the frequently used explanation of the SEC mechanism of separation by an oversimplified model of molecular sieving is not accurate. This model, however, explains quite well the separation of large particles in hydrodynamic chromatography where either very large open pores are present in the particles of column packing or the packing particles are not porous and the separation by flow is performed in the interstitial volume only.

More complicated mechanisms based on the interactions between the separated species and the stationary phase may occur in an SEC column in addition to the steric exclusion mechanism: adsorption, liquid–liquid partition, electrostatic repulsions between the separated particles and the packing material, etc. The pure SEC separation mechanism can be operating only if the column packing material and the solvent are chosen to suppress these secondary effects. If the distribution coefficient K_{sec} is larger than 1, it is certain that other interactions, e.g., adsorption, beside the steric exclusion mechanism come into play and increase the retention. Unfortunately, if K_{sec} lies between 0 and 1, it does not mean that secondary interactions are definitely not interfering. Although such interactions are secondary, they can either improve or worsen the resulting separation. From the thermodynamic point of view, the separation is carried out near equilibrium conditions and the distribution coefficient can be described by:

$$K_{sec} = \exp\left(\frac{-\Delta H^{\circ}}{RT}\right)\exp\left(\frac{\Delta S^{\circ}}{R}\right)$$

Dawkins and Hemming considered the enthalpic term on the right-hand side of this equation as a distribution coefficient, the value of which is unity, provided that size exclusion is the only effective mechanism. In such a case, the entropic term represents the pure size-exclusion mechanism. If other attractive interactions come into play ΔH° becomes negative and, if some repulsive interactions are involved, ΔH° is positive.

Other mechanisms explaining the separation in SEC have been proposed but most of them apply exclusively to the separation of macromolecules. The details can be found in the specialized literature. The above-presented approaches give an accurate basic idea of the separation of particles by SEC.

Applications of SEC

SEC allows, with respect to the basic separation mechanism, separation of particles according to differences in their effective sizes. Its application to the separation of particles in the submicron size range is limited only by the availability of column packing materials having sufficiently large pore size diameters. In order to cover as large a range of sizes of commonly fractionated particles as possible, the column packing material should have the pore size distribution from a few tenths of nanometres to hundreds of nanometres. For technical reasons, it is only possible to prepare the packings with a limited range of pore sizes and the SEC separation system is composed of an assembly of several columns in series, packed with several particle packing materials of different porosities, or another possibility is to use only one column packed with a mixture of several different packing materials with various porosities. The selectivity and the resolution of such a separation system is, however, lower than a system with a more homogeneous distribution of the pore dimensions.

Besides standard particle size separations, SEC has been successfully applied to the analytical characterization of micelles and submicron particles. Under the appropriate experimental conditions it can be used for separations in organic solvents as well as in water, at elevated temperatures, etc. An interesting application of SEC is so-called *inverse SEC*. The difference, as compared to conventional SEC, lies in the column packing particles being analysed from the viewpoint of the pore size distribution or average pore size dimensions, using a series of well-characterized size standards.

The analytical application of SEC for the determination of PSD is related to the use of either any calibration procedure and/or to the coupling of the separation system with the detector, the response of which is proportional to the size-related property of the analysed particles such as, e.g., the intensity of the

scattered light. The coupling of the concentration-sensitive detector and a size-sensitive detector, together with the use of an appropriate calibration procedure for the separation system, allows extraction of more information on PSD and other structural parameters of the particles under study.

Hydrodynamic Chromatography

Hydrodynamic chromatography (HC), as a new method for the separation of the particles of submicrometre sizes, was described by Small in 1974. HC is not a variant of SEC although some processes can participate in the separation mechanisms of both methods. It is not a subtechnique of FFF although the hydrodynamic phenomena can actively participate in the separation mechanism of FFF whose fundamental characteristic is the selective migration of the separated species due to an effective field. Formally, HC could be considered as a limiting case of FFF when the intensity of an effective external field is zero.

Principle of Separation

The name of the method designates the principal mechanism governing the separation: hydrodynamic phenomena appearing in fluids flowing through porous media or in capillaries. The separation in HC is performed in a carrier liquid flowing either through the void volume of a packed column or inside an open capillary of small diameter. The separated particles are carried by the flow with a velocity higher than the average velocity of the carrier liquid due to the tendency of the particles to concentrate in a radial position where the streamline velocity is higher compared with the average velocity of the liquid. Such a radial position corresponds to an energy minimum of the particles migrating within the field of shear forces. The driving forces which cause the radial flux of the separated particles can be of very diverse character. Another phenomenon participating in the separation processes can be the steric exclusion of the particles from a part of the volume within which the carrier liquid can flow near the column packing surface or near the wall of an open capillary. The velocity of the carrier liquid decreases to zero toward these surfaces and only small separated particles which can approach the surface of the column packing or capillary wall can elute with slow velocity in the vicinity of these surfaces. This situation is demonstrated in **Figure 11** for a model case of the HC carried out in an open capillary.

Separation Mechanisms

According to Small, the separation in HC is governed by three contributing effects: hydrodynamic forces, electrostatic repulsions, and Van der Walls forces. The density of the separated particles influences only their mobility and rotational moments. Soft particles can be deformed due to the high shear stress and this effect can influence their retention volumes.

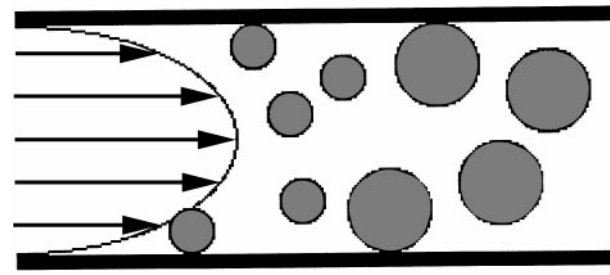

Figure 11 Schematic representation of the HC separation principle. Larger particles are excluded from the wall and can freely migrate only in a part of the volume of the capillary column. As a result, their elution times are shorter compared with the elution times of smaller particles.

A model of the separation by flow was originally proposed by DiMarzio and Guttman to explain retention in SEC. Their model approximates the structure of a packed chromatographic column to a complex system of capillaries in which the separation is caused by the same steric exclusion phenomenon as shown in Figure 11. The average velocity of the carrier liquid in a cylindrical capillary is given by:

$$\langle v(r) \rangle = \frac{\Delta P R^2}{8 \mu L}$$

where ΔP is the pressure drop along the capillary of the length L and the radius R, μ is the viscosity of the carrier liquid and r is the radial coordinate. The average velocity of uniform-sized particles is given by:

$$v_{\text{ave}} = \frac{\Delta P}{4 \mu L} \left[R^2 - \frac{(R-a)^2}{2} - \gamma a \right]$$

where a is the radius of the separated particles. The last term of the equation represents the rotational moment of the particles which reduces the velocity of their axial migration. The resulting retention is defined, similarly as in FFF, by the ratio of both velocities:

$$R = \frac{v_{\text{ave}}}{\langle v(r) \rangle}$$

Whenever HC is carried out in an open capillary, the separation is clearly dominated by this mechanism. Many authors consider that particles do not move within all the sterically accessible volume but in an

annular volume which is determined by the radial forces generated by the flow of the carrier liquid. The particles carried by the flow undergo the effect of the radial force which concentrates them within the annular volume. This force is due to the combination of the rotational and translational movements of the particles and is analogous to the Magnuson effect.

The electrostatic double layer on the surface of the separated particles influences their effective sizes. The electrostatic double layer, on the surface of the chromatographic packing or of the wall of the capillary column, reduces the accessible volume of the column due to the repulsion of separated particles of the same charge. The increased concentration of ions (ionic force) in the carrier liquid causes the screening of the surface electric charges and, consequently, reduces all electrostatic interactions. On the other hand, the reduced repulsions allow the separated particles to approach within a small distance at which the attractive Van der Walls force become effective. As a result, the hydrodynamic phenomena and electrostatic repulsions dominate the separation mechanism at a low ionic force of the carrier liquid, while at a high ionic force, the separation is dominated by hydrodynamic forces and adsorption phenomena. The order of the elution can be inverted, the particles can form aggregates, and the separation can be completely perturbed by these effects.

Applications of HC

HC is widely used for the separations of particles of very different character, starting from inorganic particles, polymer latexes, and biological cells, to synthetic and natural molecules, oil emulsions, etc. Modern short capillary columns allow substantial reduction in the separation time and an increase in the efficiency and resolution. Although HC was originally developed for the separations of micrometre-sized particles, the size range of applications has recently been lowered to tens of nanometres. The example in **Figure 12** shows the chromatogram of three polymer latex size standards separated on an open capillary column. The separation was accomplished in one minute.

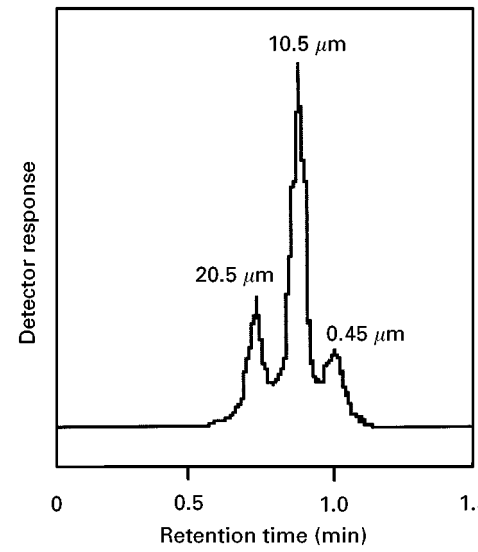

Figure 12 Separation of different size polymer latexes by HC.

Centrifugation

Starting in the early 1920s with the famous work of Svedberg, centrifugation became probably the most popular method for separation of particles. Based on extensive knowledge and experience of the sedimentation of particles in a natural gravitational field, centrifugation, using more intense inertial forces generated at slow rotational speeds, allowed the separations of relatively small particles. The invention of the ultracentrifuge (which uses extremely high speeds of rotation, allowing a reduction in the size limits of the separated species) and of new coupled detectors, upgraded a simple sedimentation fractionation technique into powerful separation methodology applicable to preparative separations as well as for analytical characterization of particles and macromolecules. The impressive progress in theory, methodology, techniques and applications was of a long-lasting nature, from the 1920s to the 1970s. Thereafter, some stagnation appeared but the beginning of the 1990 represented a renaissance era for analytical and preparative ultracentrifugation and derived techniques.

Principle of Separation

A particle suspended in a fluid settles under the effect of gravitational or inertial centrifugal force which is proportional to the effective mass of the particle, i.e., the difference between its true mass m and the mass of the same volume V of the suspending liquid, according to Archimedes principle:

$$F_1 = (m\boldsymbol{g} - \rho V\boldsymbol{g})$$

where $\boldsymbol{g}$ is the acceleration due to the gravitational or centrifugal field forces and ρ is the density of the suspending liquid. Force F_1 is opposed by the force of friction F_2 which is proportional to the velocity of sedimentation U with a constant of proportionality f, called the friction coefficient:

$$F_2 = f\,U$$

With the exception of the initial short period of time during which the sedimentation velocity of the particle increases until the steady state is reached at which both forces are equal, the velocity of sedimentation in a homogeneous liquid is constant. Stokes calculated the friction coefficient of hard spherical particles and obtained:

$$f = 6\pi\eta r$$

for a particle of the radius r sedimenting in a liquid of the viscosity η. Einstein derived the relationship between the friction and diffusion coefficients:

$$D = kT/f$$

It is evident that the sedimentation processes in homogeneous suspending liquids separate the particles according to their effective masses and if the particles are uniform with respect to their densities, the separation proceeds strictly according to the differences in particle size. The analysis of PSD can be realized on the basis of the measurement of the sedimentation velocity during the sedimentation process or from the equilibrium concentration distribution. Nevertheless, it has to be stressed that although centrifugation is, in principle, the separation method, the size-based separation of the particles can be rather complicated because various size particles sediment together and form a complex, superposed concentration gradient in which all size particles are always present in various relative proportions. On the other hand, if the separated particles exhibit nonuniformity in both size and density, size separation can be a rather difficult task.

Sedimentation processes can generate the formation of a density gradient in a complex, multicomponent suspending liquid. The particles suspended in such a density-gradient forming liquid can undergo focusing phenomena and, as a result, they can be separated according to differences in densities. Recent theoretical and experimental findings demonstrate that the size polydispersity in such cases influences the width of the focused zones. Evidently, therefore, if the particles exhibit polydispersity in size and density, the separation is complicated.

Modern theoretical approaches as well as the experimental results demonstrate that sedimentation and focusing can appear together even in a simple suspending liquid because the size polydispersity of the separated particles is itself able to generate the isoperichoric (from Greek: *isos* = equal and *perichoron* = environment) focusing phenomena. It can complicate the use of centrifugation as a simple tool for particle size separation. On the other hand, although not yet fully mastered and understood, these new approaches offer a challenge for fundamental research and development.

Separation Mechanisms

Sedimentation processes lead to the formation of a concentration gradient. Fickian diffusion, Brownian motion, general entropic tendency and repulsive interactions counterbalance the concentration gradient formed. The sedimentation of an ensemble of particles progresses until an equilibrium concentration distribution is achieved due to the opposed sedimentation and dispersive fluxes. The equilibrium can be described by the differential transport equation:

$$-D\frac{\mathrm{d}c}{\mathrm{d}x} - Uc = 0$$

where c is the concentration of the sedimenting particles and $\mathrm{d}c/\mathrm{d}x$ is the concentration gradient formed in the direction of the sedimentation. There exist some limits to the validity of this equation but the details are beyond the scope of this review. The thermodynamic approach defines the equilibrium on the basis of the chemical potential of the sedimenting species μ_i:

$$m_i(1 - v_i\rho(x))\omega^2 x\mathrm{d}x - \sum_k \frac{\partial \mu_i}{\partial c_k}\mathrm{d}c_k = 0$$

where v_i is the molar volume of the sedimenting species and ω is the angular velocity of the centrifuge rotor. The concentration distribution of uniform-size particles at equilibrium in a homogeneous liquid is exponential. When different but uniform-size colloidal particles sediment separately by forming the exponential concentration distributions, the larger size particles are compressed close to the bottom of the sedimentation cell. This situation is demonstrated in **Figure 13**. On the other hand, the sedimentation of the colloidal particles exhibiting some PSD can lead to very different equilibrium concentration distributions of the particles of different sizes. Larger size particles can be compressed closer to the bottom of the sedimentation cell but they can form focused zones at higher levels as well. These two situations are demonstrated in **Figure 14**.

In the first case shown in Figure 14, two exponential concentration distributions corresponding to two different size particulate species are superposed. The lower part of the sedimentation cell contains a higher proportion of larger particles compared with the original mixture and vice versa for the upper part

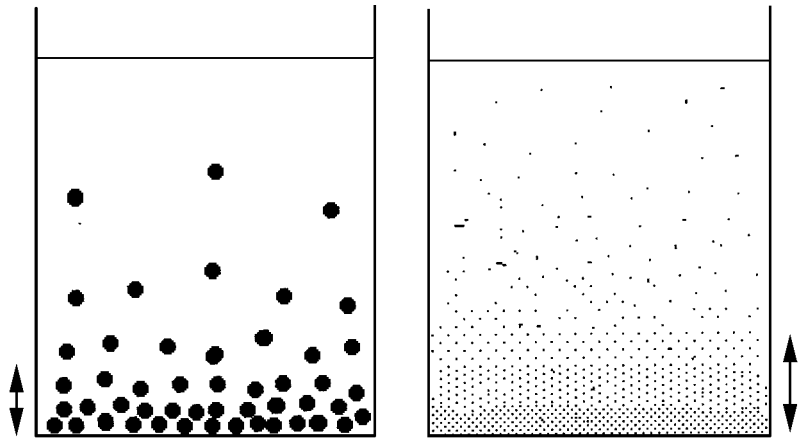

Figure 13 Schematic representation of the sedimentation of different size particles. Concentration distribution is more compressed to the bottom of the sedimentation cell for larger size particles (left) and centre of gravity of the concentration distribution is closer to the bottom compared with smaller size particles (right).

of the sedimentation cell, and thus size fractionation exists. It is impossible, in principle, to achieve more complete size separation of particles by simple centrifugation.

In the second case shown in Figure 14, larger particles are focused in the density gradient due to the equilibrium exponential concentration distribution of smaller particles. The concentration distribution of larger focused particles approaches a Gaussian distribution function.

The two imaginary cases shown in Figure 14 demonstrate two limit situations which can appear in actual centrifugation experiments in a homogeneous suspending liquid. The focusing phenomenon is, of course, actively exploited in isopycnic (or more generally isoperichoric) focusing separations of particles. In such cases, a two- or multicomponent liquid is used to form the density gradient and larger particles are separated according to density differences.

The particle–particle interactions which limit the degree of freedom of the particle movements, and whose importance increases with increasing concentration, are the major factors imposing the particular concentration distribution of each sedimenting species of a polydisperse colloidal sample. Consequently, the results of the particle separation performed by any centrifugation method must be carefully evaluated.

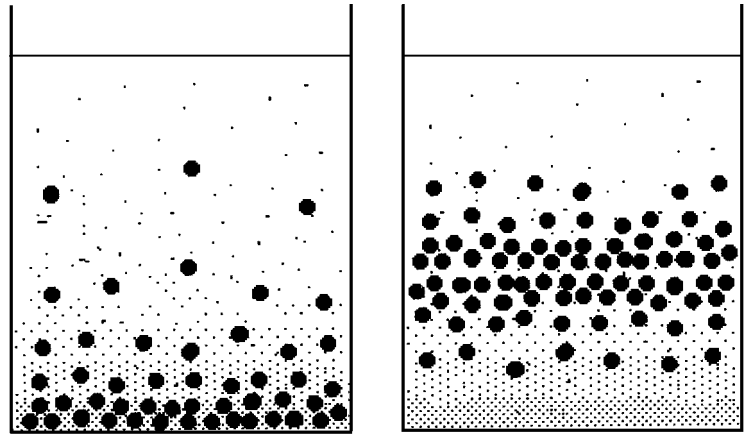

Figure 14 Schematic representation of the sedimentation of a mixture of different size particles. The exponential concentration distribution of larger and smaller size particles can be either superposed (left) or larger size particles can be focused within the density gradient formed by the exponential concentration distribution of smaller particles (right).

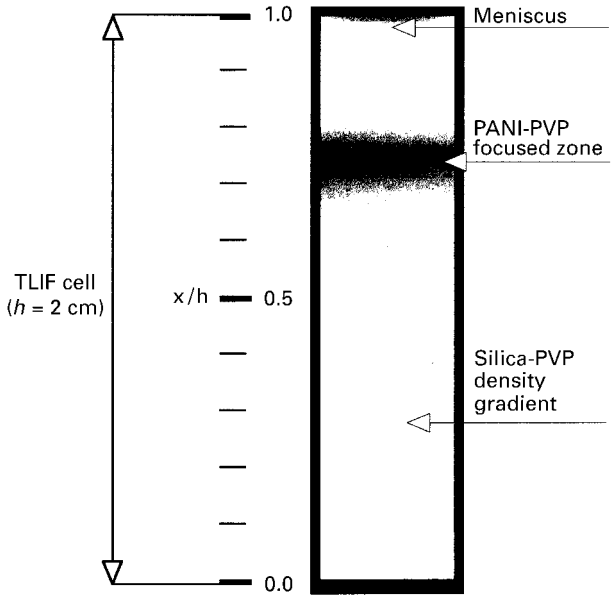

Figure 15 Isoperichoric focusing of coloured polyaniline particles in the density gradient formed by colourless silica particles in thin-layer isoperichoric focusing (TLIF) cell in a centrifugation experiment.

Applications of Centrifugation

When taking into account the potential and limitations of sedimentation processes, centrifugation can be successfully applied and, in reality, is widely used for the separation of colloidal particles of very different character: inorganic, polymer and biological, and also for the separations of macromolecules. An example of the use of centrifugation is in **Figure 15** which shows the zone of the coloured polyaniline particles focused from a bidisperse mixture with colourless silica particles. This focusing experiment was, indeed, intended not to separate the polyaniline particles from the silica particles of comparable size but to prove the existence of the focusing phenomenon under the given experimental conditions. However, the size separation of the particles using this phenomenon is real.

Electrophoresis

Electrophoresis is a separation technique based on differential transport of electrically charged species. The discovery of electricity was paralleled with an understanding of electrophoretic phenomena and consequently, this separation technique can be considered as classical.

Over the last two decades, all electrophoretic techniques have undergone an explosive growth, especially as concerns the analytical applications of the capillary version of electrophoresis. Nevertheless, there are only few publications describing the applications of this technique to the separation of charged particles. This can be explained by the fact that separation in electrophoresis is primarily based on differences in electric charge density which is inherently related to the size of the separated particles. As described in the section on HC, the effective size of the particles includes the thickness of the electric double layer which varies with the ionic force of the suspending liquid. This means that whenever the size separation concerns the particles in their natural environment, their effective size includes the electrostatic double layer and separation can be carried out by using electrophoretic transport processes. As the electric charge contains information on the nature of the particle surface, separation by electrophoresis is certainly a useful technique when used appropriately.

The general theory of electrophoretic separations applies to particle separations as well. This has been discussed above in respect to electric FFF. As the applications of electrophoretic techniques to particle separations are still very limited, it is impossible to review this technique as fully as for the other separation methods. Only one recent example, an interesting separation of polyaniline and silica particles, is shown in **Figure 16**.

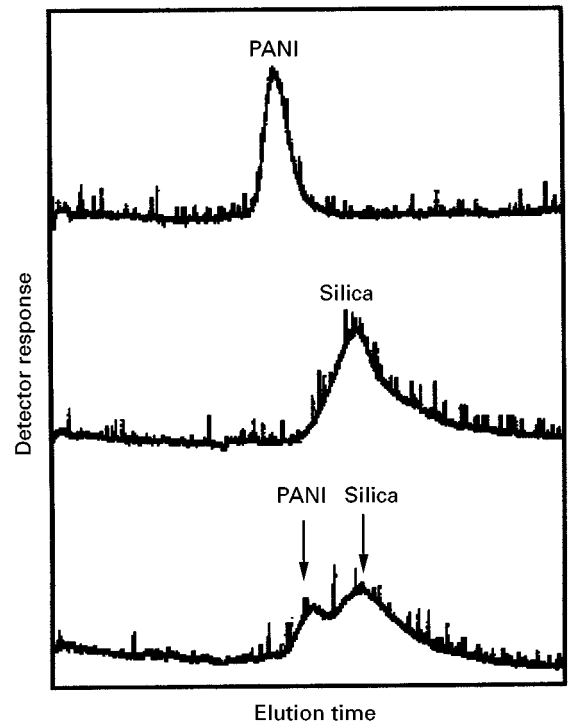

Figure 16 Electropherograms of the individual polyaniline (PANI) and silica composite particles and of the separated mixture of both obtained by capillary electrophoresis.

Future Development

A search for the historical origins of a scientific discovery is often a difficult task but, with regard to the rapid advances in separation science in general, and of particle size separations in particular, the long-term prediction of progress is almost a 'mission impossible'. However, cautious examination of the state of the art and of potential exigencies concerning particle size separations, allows a few statements about what is likely to happen in the near future to be made.

Further increases in efficiency, resolution and selectivity represent a permanent challenge in particle size separations. An ideal is to separate two particulate species differing by a minimal increment in terms of a 'construction' unit, e.g., one molecule or atom, and not only in terms of 'size increment' which is a rather arbitrary choice.

Increase of the separation speed can be an important factor whenever the separated particles exhibit an evolution in time and it is necessary to capture information on the actual PSD at a given moment. Many biological concepts are approached in this way. The ways to be explored lead to more extensive use of supercritical fluids allowing substantial increase of transport coefficients.

Most recent methods and techniques of particle size separations exploit simple physical and physicochemical principles single driving forces leading to the separation. Coupling of two or more physical fields and field gradients as selective driving forces and their combinations with nonselective transport due to the carrier fluid flow seems to be a recently emerging approach.

Large-scale particle size separations represent important parts of many industrial technologies. The performance of a large-scale separation is often lower in comparison with an essentially identical technique applied under analytical-scale conditions. The optimization of large-scale separation processes in order to approach the performances comparable with analytical-scale conditions. The optimization of large-scale separation processes in order to approach the performances comparable with analytical-scale separations has a potentially important economic impact.

The permanent search for noninvasive conditions in particle size separations is an important field of activity related to fundamental research in the life sciences and also to many important biotechnologies.

These directions of potential future progress in the domain of particle size separations are certainly not exhaustive but they represent an overview of, probably, the most important activities in research and development.

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