

Fractionation and characterization of nano- and microparticles in liquid media

Petr S. Fedotov · Nataliya G. Vanifatova ·
Valery M. Shkinev · Boris Ya. Spivakov

Received: 2 December 2010 / Revised: 17 January 2011 / Accepted: 18 January 2011 / Published online: 12 February 2011
© Springer-Verlag 2011

Abstract Submicron and micron particles present in liquid environmental, biological, and technological samples differ in their dimensions, shape, mass, chemical composition, and charge. Their properties cannot be reliably studied unless the particles are fractionated. Synthetic particles applied as components of analytical systems may also need preliminary fractionation and investigation. The review is focused on the methods for fractionation and characterization of nanoparticles and microparticles in liquid media, the most representative examples of their application, and the trends in developing novel approaches to the separation and investigation of particles. Among the separation techniques, the main attention is devoted to membrane filtration, field-flow fractionation, chromatographic, and capillary electrokinetic methods. Microfluidic systems employing the above-mentioned and other separation principles and providing a basis for the fabrication of lab-on-chip devices are also examined. Laser light scattering methods and other physical techniques for the characterization of particles are considered. Special attention is given to “hyphenated” techniques which enable the separation and characterization of particles to be performed in online modes.

Keywords Nanoparticles · Microparticles · Separation · Characterization · Membrane filtration · Field-flow fractionation · Chromatography · Electrokinetic methods · “Hyphenated” techniques

Abbreviations

AF4	Asymmetrical flow field-flow fractionation
CTFFF	Coiled-tube field-flow fractionation
CZE	Capillary zone electrophoresis
DEP	Dielectrophoresis
DLS	Dynamic light scattering
FIFFF	Flow field-flow fractionation
FFF	Field-flow fractionation
GE	Gel electrophoresis
GPC	Gel permeation chromatography
HDC	Hydrodynamic chromatography
HPLC	High-performance liquid chromatography
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
IEF	Isoelectric focusing electrophoresis
ITP	Isotachophoresis
LC	Liquid chromatography
LIF	Laser-induced fluorescence
MALLS	Multangle laser light scattering
MMF	Multistage membrane filtration
SAXS	Small-angle X-ray scattering
SdFFF	Sedimentation field-flow fractionation
SDS	Sodium dodecyl sulfate
SEC	Size-exclusion chromatography
SPLITT	Split-flow thin-cell
TFFF	Thermal field-flow fractionation

P. S. Fedotov (✉) · N. G. Vanifatova · V. M. Shkinev ·
B. Y. Spivakov
Vernadsky Institute of Geochemistry and Analytical Chemistry,
Russian Academy of Sciences,
19 Kosygin Street,
119991, Moscow, Russia
e-mail: fedotov_ps@mail.ru

Introduction

Characterization of nanoparticles and microparticles is an important challenge in analytical, environmental, biological, and nanomaterial sciences. The size distribution of

particles may strongly affect the characteristics of various materials. Furthermore, particles of different size frequently have different chemical composition and properties that cannot be conveniently studied unless the particles are fractionated.

By convention, particles may be arranged into three main groups: environmental, biological, and synthetic (Fig. 1). For the last 20 years the fractionation and analysis of environmental colloids and solid particles related to inorganic compounds and nonliving organic matter have been extensively discussed in the literature [1–3]. It has been ascertained that different substances may occur in environmental systems as freely dissolved molecules or ions or may be associated with larger macromolecules, colloids, or solid particles. For instance, trace metals and metalloids can be bound to humic substances or oxyhydroxides of iron, aluminum, and manganese. To estimate the behavior and toxicity of contaminants, particles of different nature and size should be fractionated before their analysis. Separation of biological particles is a special task that is very significant for biology, biochemistry, and medicine. The fractionation and investigation of synthetic submicron particles is becoming more and more relevant owing to the rapid development of nanotechnology. It is known that the size and structure of nanomaterials are responsible for their novel properties (electric, chemical, magnetic, optical, and mechanical). Therefore, characterization of nanomaterials has become the subject of intense research both from the theoretical point of view and for their potential application. For example, the sizes and shapes of metal nanoparticles are important factors that determine their properties as chemical catalysts, adsorbents, biological stains, and elements of novel nanometer-scale optical, electronic, and magnetic devices.

Particles applied in analytical chemistry may also need preliminary fractionation and characterization. Nanoparticles and microparticles can be used in:

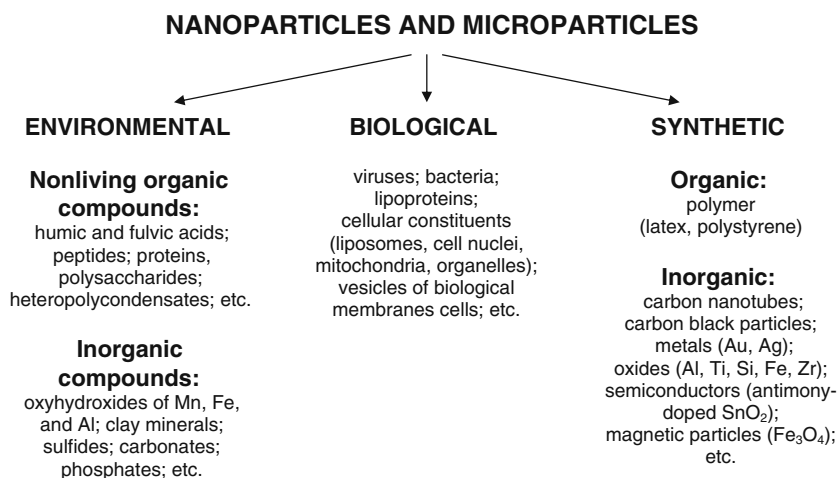
- Solid-phase extraction (carbon nanotubes, iron oxide nanoparticles as adsorbents)
- Micellar extraction
- Chromatography
- Capillary electrophoresis and capillary electrokinetic chromatography (polymer and silica nanoparticles as the pseudostationary phase, covalent and dynamic coating of capillary walls with silica, Au/TiO₂ and gold nanoparticles)
- Other analytical techniques

A number of techniques employed for fractionation and characterization of particles in liquid media exemplify the relevance and difficulty of this problem. Our review is focused on the most representative methods, examples of their application, as well as some trends in developing novel approaches to the fractionation and investigation of particles. Special emphasis is given to “hyphenated” techniques which enable the separation and characterization/analysis of particles to be performed in online mode.

Fractionation of nanoparticles and microparticles

In general, particles bigger than about 50–100 μm can be fractionated by conventional wet (or dry) sieving [4, 5]. Membrane filtration, field-flow fractionation (FFF), and split-flow thin-cell (SPLITT) fractionation can be used to separate particles in the size range from 1 to 100 μm. It should be noted that techniques such as sedimentation and elutriation, which have been known for centuries, are also applied to the separation of microparticles. Gel chromatog-

Fig. 1 Origin and types of nanoparticles and microparticles



raphy, membrane filtration, FFF, and capillary electrophoresis can be employed to fractionate solid and colloidal particles smaller than 1 μm into classes based on their size, density, or charge [4]. Working size ranges for some representative separation techniques are shown in Fig. 2. Among novel approaches that are being developed, microfluidic techniques [6], photophoresis [7], and hydrophoresis [8] should be mentioned.

Membrane methods

Ultrafiltration and microfiltration are pressure-driven membrane transport processes used to separate macromolecules, colloids, microorganisms, and solid particles from fluids. Generally, “microfiltration” refers to processes used to remove particles larger than 0.02–0.5 μm . For ultrafiltration of macromolecules and colloidal particles, membrane filters of 0.002–0.5- μm pore size are available.

Ultrafiltration and microfiltration have been successfully employed in analytical chemistry [9]. The possibility of preconcentration and separation of different species without separating agents is one of the advantages of some membrane techniques [9–11]. This is of particular interest in chemical analysis to decrease the probability of contamination, as well as in biological and clinical assays where the particles to be analyzed should not suffer changes during their prepreparation.

Membrane filtration as compared with other separation methods makes it possible to work with large volumes of samples up to 1 m^3 [9]. For example, the technique can be used for preconcentration of nanocolloid plutonium during analysis of seawater. Another advantage of membrane

filtration is the possibility to interpret the results of separation of particles and macromolecules without using size reference materials because the membrane pore diameters themselves can serve as size standards. This feature enables membrane filtration and its combinations with other fractionation methods to be used for preparing size reference materials.

Membrane filtration shows considerable potential for the separation of nanoparticles into the corresponding fractions, the separation of a polydisperse sample into fractions of differing mean core diameter, and the purification of thiol-stabilized 3-nm gold nanoparticles [12]. NMR, thermogravimetric analysis, and X-ray photoelectron spectroscopy measurements demonstrated the possibility to obtain nanoparticles with a much higher degree of purity than is possible by dialysis or a combination of solvent washes, chromatography, and ultracentrifugation [12]. UV–vis spectroscopic and transmission electron microscopic analyses show that membrane filtration offers the ability to separate nanoparticles of disparate core size. Membrane filtration demonstrated applicability to rapid preparation of high-purity gold nanoparticles and to size separation of heterogeneous nanoparticle samples [12].

The results of membrane filtration may be distorted owing to the following physicochemical artifacts:

- Aggregation of particles and colloids at the membrane surface that can result in gel formation
- Adsorption of compounds of any size on the membrane surface and pore walls
- Coagulation at the membrane surface due to concentration polarization [13–15]

For minimization of artifact effects, the modification of the membrane surface or more effective organization of solution flow is used. For modification of the membrane surface, chemical methods are usually applied. Some new approaches are based on geometrical modification of membrane pores [16] or modification of the membrane surface with nanoparticles [17]. A proposed tangential flow ultrafiltration method was compared with the widely used ultracentrifugation method for efficiency in concentration, size selection, and minimization of the aggregation state of silver nanoparticles. The tangential flow ultrafiltration method proved to be more efficient [13].

The ability of cross-flow filtration to perform correct size fractionation of natural aquatic colloids (materials from 1 nm to 1 μm in size) and particles (larger than 1 μm) has been evaluated using scanning electron microscopy combined with atomic force microscopy [18]. Other membrane cross-flow techniques were used to study the element speciation in waters of different type [19, 20]. In most

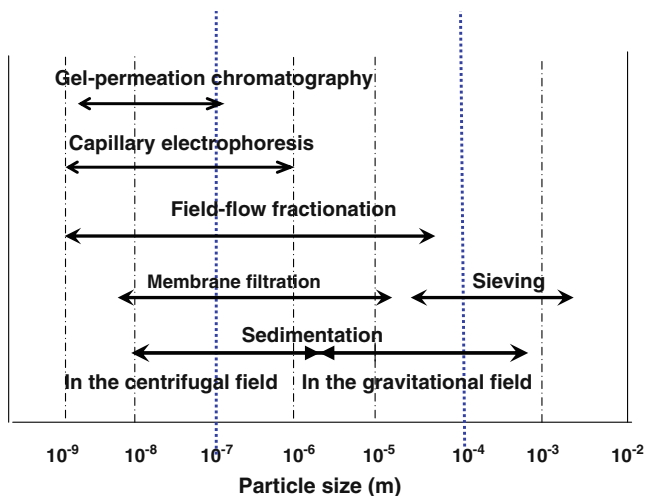


Fig. 2 Fractionation of nanoparticles and microparticles. Working size ranges for some representative separation techniques

cases, *online multistage membrane filtration* (MMF) with tangential flow enables the problems of membrane filtration to be resolved [21–23]. In multistage separations, minimization of concentration polarization, membrane fouling, and loss of separated components, which can occur in a cascade of stirred cells or hollow-fiber modules owing to the use of relatively lengthy tubing and a number of connectors and valves, is achieved by using MMF. Specially designed online MMF systems have been employed for fractionation of particles and macromolecules and for speciation studies of metal and nonmetal ions bonded with low molecular weight and macromolecular organic matter, or adsorbed on solid particles of different size [21–24]. The MMF device consists of several coupled-together discs in which different membranes with a pore size from 8 μm to 50 kDa are inserted. The discs have fine channels in the upper part and in the lower part a drainage system connected to the undermembrane space of next disc. The sample solution is fed from a reservoir to the first (lowest) filtration step, where it partially moves in parallel to the membrane surface (tangential flow). The filtrate penetrates the chamber (reservoir) of the next (upper) disc, is partially filtered, and is partially recirculated; the process repeats at each next step with a membrane of smaller pore size. Therefore, both particulate and dissolved components of each fraction remain in aquatic media after the filtration run and thus are taken for subsequent analyses.

The use of MMF employing semipermeable nanofilters, ultrafilters, and microfilters enables separations that cannot be performed using other means [9]. Since MMF minimally disturbs the initial equilibrium in the liquid–solid systems under study, membrane-based size fractionation is useful for determination of the distribution patterns of trace elements in natural waters and other fluids. Elements associated with suspended particles, colloids, and polymers as well as the ionic forms of these elements can be determined after membrane separation and preconcentration. For example, multistage size fractionation of aquatic components using membranes and its combination with inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), and other analytical detection methods can be used to:

- Separate particles of various types and macromolecules from each other for further studies
- Study to what extent trace metals and other compounds are associated with these macromolecules and particles
- Estimate the size of macromolecules, colloids, and particles
- Determine the equilibrium constants for the distribution of different compounds between particles of different size and water

The fractionation systems and schemes have been tested on different waters, including polluted water from environmentally important sources [21–24].

The membranes are also necessary to filter viruses (0.03–1 μm) and bacteria (0.5–20 μm). Size cutoffs for microfiltration are usually expressed in terms of micrometers, whereas ultrafiltration cutoffs are often expressed in terms of molecular mass. It should be noted that the concentration of viruses in water is much lower than that of bacteria; therefore, their preconcentration and separation from other suspended matter is important in virological analysis. Viruses may be enriched by ultrafiltration by use of tangential flow or hollow-fiber filtration units, most suitable for preconcentration from large-volume water samples. Another possibility consists in adsorption of the viruses on the microfilter matrix followed by elution with diluents containing cell extracts, serum, or surfactants [9].

Membrane filtration is a rather versatile method [1]. However, the separation of nanoparticles and microparticles is performed mainly according to their effective size. Hence, particles having different density and surface properties can be found in one fraction.

Field-flow fractionation

The general concept of FFF was developed in the 1960s by Giddings [25]. FFF is a set of liquid chromatography (LC)-like elution methods. However, unlike chromatography, FFF requires no stationary phase and only physical interactions are involved in the separation process [26]. The retention and elution of colloidal and particulate matter are achieved by a combined action of the nonuniform flow velocity profile of a carrier fluid and a physical force field (gravitational, centrifugal, electric, etc.) applied at a right angle to the thin (0.05–0.5-mm) channel. The FFF theory assumes three major separation mechanisms, namely, *normal* (*Brownian*), *steric*, and *hyperlayer* (*focusing*) elution modes [27].

Normal FFF is applicable to submicron particles (smaller than 1 μm) and requires a specific sample injection procedure described in the literature as *stop-flow injection* or a *relaxation step* [28]. Relaxation time is needed to equilibrate the sample under the influence of a force field in the absence of flow. The injected sample is forced to concentrate in a layer at a certain position on the accumulation wall. The effect of concentrating and diffusion processes leads to an exponential distribution of particulate matter. When the flow is switched on, the elution begins with smaller particles, which are distributed closer to the center of the channel in faster flow streams; larger particles appear later. Two main factors influencing the sample behavior in *Brownian* elution mode are the properties of particles characterized by the

diffusion coefficient and the strength of the cross-field applied [27, 28].

Steric elution mode is observed for micron-range particles (larger than 1 μm). In this case the elution order is inverted and larger particles move ahead of smaller ones [29]. It is considered that particles are in touch with the accumulation wall during the entire migration time and consequently larger particles are situated closer to the center of the channel in faster flow streams. In this case the retention is independent of the field applied and the flow rate.

In fact, the retention of particles bigger than 1 μm typically increases with flow rate. It has been suggested that particles are driven away from the wall by a hydrodynamic lift force and elevated into faster flow streams. [30]. The particles form thin layers at an equilibrium position where the external field is balanced by the opposite hydrodynamic lift force. This separation mechanism was named *hyper-layer (focusing)* mode.

Sometimes the experimental data do not fit to classical retention equations and other assumptions are required. The deviations may arise because the following factors are usually neglected: steric exclusion, lift forces, particle slip, van der Waals forces, electrostatic forces, nonparabolic flow (edge effects and viscosity gradients), and nonuniformity of the field across the channel thickness [31]. Hence, in most cases FFF systems need experimental calibration using standard monodisperse samples of spherical particles [32].

FFF comprises a family of subtechniques employing force fields of different nature [27]. *Gravitational FFF* is the simplest subtechnique. It employs Earth's gravitational field to fractionate particulate matter in a micrometer size range (microorganisms, blood cells). A similar subtechnique is *sedimentation FFF* (SdFFF), which uses a centrifugal force field. The fractionation of micron-sized particles of different origin (inorganic colloids, pigments, viruses, liposomes, ribosomes) is performed in a circular flat channel inserted inside a centrifugal basket [27]. SdFFF is considered to be an appropriate and powerful method for cell separation [28]. Recent studies have shown that SdFFF can be successfully applied to the fractionation and characterization (sizing) of submicron particles starting from 50 nm: industrial carbon blacks [33], silica nanoparticles [34, 35], silver nanoparticles [36], gold nanoparticles [37], etc.

The oldest FFF subtechnique is *thermal FFF* (TFFF). The field force in this case is a temperature difference across the channel. One side of the channel is heated and the other side is cooled. A relatively small temperature difference between the top and bottom walls of the channel provides a high thermal gradient. TFFF has been used for the separation of linear polystyrenes, lyophilic polymers,

materials in the refinery industry, polyvinyl acetate mixtures containing ultrahigh molecular weight polymers and microgels [27, 38], and other natural, biological, and synthetic polymers [39]. The effects of chemical composition on the retention are sufficiently strong, and TFFF can be readily used for the separation of equal-size particles of different composition [40]. One of the disadvantages of TFFF is a possible flocculation of the dispersed system. Besides, classical macroscale TFFF instruments require long separation times. A few years ago the miniaturization of TFFF was proposed [41, 42]. Micro-TFFF can be described by steric and hyperlayer elution mechanisms, depending on the flow rate [43]. Micro-TFFF allows one to increase significantly the temperature gradient, reduce the dead volume of the system, and improve the separation [27, 44, 45].

The most versatile FFF subtechnique is *flow FFF* (FIFFF), employing a nonspecific hydrodynamic force field across the channel, which is generated by a secondary mobile phase cross-flow. The sample components are driven by the cross-flow toward the accumulation channel surface (permeable membrane). The positions of individual species in the laminar carrier fluid profile correspond to their diffusion coefficients and the separation is based on differences in these coefficients. Selection of an appropriate membrane depends on the properties of the macromolecules or particles to be separated; the pore size should be sufficiently small to retain components but large enough to allow the carrier fluid to pass through. The use of flat and smooth membranes is required because any membrane flaws would affect the separation process. The proper selection of membranes is still the major problem of FIFFF owing to possible interactions with the suspension components and membrane-induced nonuniformity in the channel thickness [27]. Regenerated cellulose is a widely used material for FIFFF membranes. FIFFF separates on the basis of the effective size of the molecules or particles, and the process is independent of density, whereas SdFFF separates on the basis of buoyant mass, i.e., size and density [2].

There are two most important types of FIFFF. In *symmetrical FIFFF*, cross-flow is achieved by pumping the carrier fluid directly across the channel through porous frits. Both channel walls are permeable, but the accumulation wall is covered by an ultrafiltration membrane impermeable to the sample components [46]. This technique can be applied to the size fractionation of different synthetic polymers, liposomes, proteins, viruses, dissolved organic matter, humic and fulvic acids as well as their complexes with trace metals, diesel soot particles [2], marine colloids [47], heterogeneous and arbitrarily shaped natural colloidal particles [48], gold colloid standards starting from 5 nm [49], etc.

In *asymmetrical FIFFF* (AF4) only one channel wall is permeable. A trapezoid shape of the separation unit has been developed for AF4 to avoid constant loss of axial flow of the carrier fluid along the channel [27]. In general, the channel geometry affects significantly the separation efficiency in AF4. Three different channel designs were evaluated using polystyrene latex nanoparticles [50]. The channel breadth was held constant for one (rectangular) profile, and was reduced either linearly (trapezoid profile) or exponentially (exponential profile) along the length for the other two channels. Compared with a rectangular channel, trapezoid and exponential profiles provided better performance of AF4 and allowed band broadening to be reduced [50].

Currently, AF4 has become one of the most favored FFF systems [27]. It has been applied to the fractionation and sizing of carbon nanotubes [51], humic substances [52], natural aquatic colloids [53], polysaccharides [54], synthetic polymers [55], gelatin nanoparticles in drug carrier systems [56], aqueous C₆₀ fullerene aggregates [57], virus-like particles [58, 59], etc. Besides, AF4 provides a reliable basis for developing different “hyphenated” techniques for separation, investigation, and analysis of colloidal and particulate matter in liquid media. This will be discussed in detail later.

Other FFF subtechniques (*magnetic FFF*, *acoustic FFF*, *pressure FFF*, *dielectrophoretic FFF*, *photophoretic FFF*) are more specific and can be applied to a rather limited number of particulate sample types [27]. Acoustic FFF is a particular technique that is worthy of consideration. Ultrasound is a type of energy which can help analytical chemists in various laboratory tasks, from cleaning to determination [60]. Some promising experimental approaches to particle separation in a megahertz ultrasonic standing wave field and the theory of the separation processes have been reported [61]. As in other FFF techniques, an external field (acoustic) can be applied perpendicular to the flow direction. A method has been described for fractionating suspensions on the basis of the transient response of fine particles (0.1–100 μm) to a planar, ultrasonic wave field applied across the thin dimension of a long rectangular channel [62]. The method was used for the continuous fractionation of polystyrene particles in aqueous suspensions.

However, particles having a density different from that of a surrounding medium are subject to sedimentation, which makes it difficult to accomplish stable separation with the arrangements described above. This led to the idea that the acoustic field should be applied vertically, and in that case one can couple a gravity field with an acoustic field [61, 63]. Such an approach and corresponding devices were used for the fractionation of microparticles of alumina, silica gel, quartz powder, and polystyrene latex as well as

for the separation of plant cells and other particles of biological origin.

In general, FFF is a powerful and versatile fractionation and sizing method. The size range covered is from about 1 nm to 100 μm . The main limitation of FFF is the weight of the particulate sample that can be handled, which is less than 1 mg. This requires an increased sensitivity of the detection techniques used for the analysis of separated fractions. Besides, samples should be highly homogenized to provide representative data.

Fractionation in a rotating coiled column, which can also be attributed to FFF, enables the sample handling weight to be increased up to 1 g [64–66]. This technique, named *coiled-tube FFF* (CTFFF), employs a complex asymmetrical force field generated in planetary centrifuges [67]. Among the other FFF techniques, CTFFF is more similar to SdFFF utilizing a circular channel inserted inside a centrifugal basket. Although both SdFFF and CTFFF are based on the centrifugal force field, there are two important differences between these techniques. First, in the case of CTFFF, the mixture to be separated is not introduced into a thin channel but is pumped with the carrier fluid through a long rotating coiled column (inner capacity about 20 mL). Second, in the planetary centrifuge used for performing CTFFF, particles and fluid in the coiled tube are under the action of the complex asymmetrical centrifugal force field. This field is dependent on the ratio of rotation and revolution radii (β value). Fractionation and retention of solid particles (size range 100 nm to 200 μm) as a function of β , column rotation speed, carrier fluid composition, and flow rate have been studied taking as examples latex beads, silica gel, and quartz sand particles with an irregular geometry [66, 68]. CTFFF has also been applied to the speciation analysis of environmental solids. Silt, clay, and sand fractions were successfully separated from soils. It should be noted that rotating columns can be also used for the sequential flow-through extraction of trace elements from solid samples. Determining elements in each size fraction enables a detailed pattern of the distribution of toxic elements in soils to be obtained [69].

Split-flow thin-cell fractionation

This technique is similar to FFF [70]; however, like CTFFF, it enables the handling of large quantities of samples up to gram levels. Stream splitters are inserted into the flat channel, so the SPLIT system has two inlets at one side of the channel and two outlets at the other side. The separation is achieved by the combined action of controlled flow rates and a gravitational cross-field. A thin layer of the sample is formed close to the upper wall of the channel; its thickness is equal to the distance between the inlet splitting plane and the upper channel wall. Near the channel outlets, the outlet

splitting plane is formed; its lateral position depends on the ratio of the upper and lower exit flow rates. SPLITT systems and related techniques were successfully applied to the fractionation and investigation of micron and submicron particles such as aquatic colloids [71], sea sediments [72], and polymer beads [73, 74]. Acoustic programming in SPLITT fractionation of latex particles [75] and splitterless systems for the separation of sea sediments [76] were also reported. It should be kept in mind that when SPLITT techniques are used, only two fractions can be recovered in one experimental run (e.g., particles larger than 1 μm and smaller than 1 μm). Thus, for the separation of a number of different fractions multistep procedures are required.

Chromatographic methods

A large variety of chromatographic methods can be successfully applied to the fractionation of nanoparticles and microparticles of various types and origins.

Gel permeation chromatography (GPC), also known as *size-exclusion chromatography* (SEC), has been developed especially for the separation of macromolecules and particles. The sorting by size is achieved by using a porous matrix. Smaller particles are retarded because they can access a larger pore volume of the packing column. GPC can be used for size separation of CdS, ZnS (2–20 nm), silica colloids [77], dextran-modified iron oxides [78], chiral gold clusters covered with 1,1'-binaphthyl-2,2'-dithiol [79], magnetic nanoparticles, etc. Choosing and optimizing the mobile phase composition may play a key role in the fractionation procedure. Gold nanoparticles [80, 81] and Au/Pd core-shell nanoparticles [82] in the size range from 10 to 80 nm were separated using sodium dodecyl sulfate (SDS) solution to stabilize the particles. Shape-based separation of rod-like and spherical gold nanoparticles was achieved by adding SDS and Brij-35 to the eluent [83]. Size separation of quantum dots and nanoparticles was performed using a concentrated poly(methyl methacrylate) brush [84]. This polymer exhibits very good repellency for particles that is not dependent on their surface nature.

A GPC subtechnique, *recycling GPC*, was applied to the separation and characterization of gold nanocrystals stabilized by thiols [85]. Alternate recycling was shown to provide improved efficiency and high resolution compared with closed-loop recycling. The resolution ratio of nanocrystal separation was found to increase with the square root of the cycle number, in good agreement with the theory. As a result, baseline separation of nanocrystals which differ in size by only 6 Å has been achieved.

Hydrodynamic chromatography (HDC) was proposed by Small [86] as a technique for size fractionation of colloidal and solid particles in a size range from a few nanometers to

a few micrometers. There are two subtechniques of HDC: *packed column chromatography* and *capillary tube (open tubular) HDC*. Particles are injected into a mobile phase at a known flow rate either through a column packed with nonporous uniform spheres or through a long capillary tube. The particle migration rate depends on the particle size, the size of the packing material or capillary tube, and the ionic composition of aqueous phase. In either case, variation of flow across the interstitial voids (in the case of the packed column method) or across the capillary (capillary tube method) causes larger particles to travel faster, and smaller particles to travel more slowly [3]. HDC was applied to the separation and size characterization of synthetic polymer particles, biopolymers, and lipid nanocapsules [87]. Packed columns were used for size classification and purification of porous ethyl-bridged hybrid packing materials (particles) in the micron to submicron range [88]. Subsequent chromatographic evaluation of classified packing material showed significant improvement in column performance and decrease in flow resistance over the unclassified material.

Wide-bore HDC employs capillaries with inner diameter larger than 0.7 mm. The method is based on the radial distribution of the particles in the capillary due to the hydrodynamic flow profile and diffusion coefficients of the particles depending on their size. A wide-bore polyether ether ketone capillary was applied to CdS and gold sols in the size range between 3 and 27 nm [89]. Subsecond separation of fluorescein and polystyrene latexes based on their passage through an open wide-bore capillary was demonstrated [90].

A series of chromatographic methods that are traditionally used for the separation of solutes can also be applied under certain conditions to the separation of nanoparticles. Monolayer protected gold clusters (smaller than 2 nm) were separated by *high-performance LC* (HPLC) and detected electrochemically [91, 92]. HPLC separation of polydisperse gold nanoclusters protected with monolayers of *N*-acetyl-L-cysteine was achieved on a C₁₈ column using a gradient elution program [93]. *Reversed-phase HPLC* was used also for size-dependent separation of Au/Pt core-shell nanoparticles (5.3–40.1 nm) on a porous silica-based column with SDS in deionized water as the mobile phase [94]. Charged polydisperse gold nanoparticles protected by monolayers of *N*-acetyl-L-cysteine and tiopronin ligands (1–2 nm) were also fractionated by *ion-pair chromatography* [95]. *Countercurrent chromatography* based on the retention of one phase of a two-phase liquid system in a rotating coiled column was applied to the size separation of silver nanoparticles modified by carboxylate anions (10.5–21.1 nm) using a step-gradient extraction process [96]. Particles were extracted into the organic phase (toluene/hexane 1:1, v/v) with tetraoctylammonium bromide, owing

to ion-pair adduct formation. Smaller nanoparticles were found to be more readily transferred to the organic phase than larger ones. Compared with the stepwise extraction, the step-gradient extraction provided much better size discrimination.

Electrokinetic methods

These methods have been extensively applied to the separation of nanoparticles. *Gel electrophoresis* (GE) can be regarded as an important tool in nanotechnology and nanobiotechnology. The technique is based on the differences in species migration rates in a gel under an applied electric field. Gel density is an important parameter to be taken into account. It may affect the migration rate in the gel or even discriminate very large species. Therefore, the gel density should be selected according to the particle size. There are planar slab and column GE modes. The mobility depends on the charge, size, and shape of a particle. Although some work was done in the field of nanoparticle separation by polyacrylamide gel electrophoresis [97], mainly agarose gel electrophoresis has been applied. The preference for agarose over polyacrylamide can be ascribed to the easy implementation of this technique and to the wide mean pore diameters easily obtainable. The mean pore size in the gel was found to be dependent on the concentration of added agarose (typically 0.1–5%).

GE size-based separations of unmodified and modified polystyrene and gold conjugates, silver, Fe₂O₃, CdSe/ZnS, and CdTe nanoparticles were performed [98]. Size separation and shape (spheres, rods, plates) separation of gold [99, 100] and gold and silver nanoparticles coated with a charged polymer layer were demonstrated [101]. The particle shape was shown to determine a trend of varying electrophoretic mobility with size. Smaller spherical particles and short rods experience less resistance from the gel and move faster. Separation of nanogold conjugates with a different number of bound oligonucleotides can be achieved in agarose gel [102]. GE can also be applied to preparative and semipreparative fractionation of gold nanoparticles (3.5–18 nm) [99]. This allows one to study off-line fractions corresponding to different electrophoretic zones.

Capillary GE employing linear polyacrylamide as the sieving medium was used for highly effective size separation of Cd/Te quantum dots (1.9–4.5 nm) stabilized with 3-mercaptopropionic acid [103]. Resolution was shown to increase with increasing polymer concentration. Improvement of capillary GE resolution by addition of poly(ethylene oxide) to the carrier electrolyte was demonstrated for nanocomposites of Cd/ZnS with different proteins [104].

Capillary zone electrophoresis (CZE) enables nanoparticles to be separated owing to differences in their

electrophoretic mobility. Therefore, particles that differ in size and/or zeta potential can be discriminated. Zeta potential, pH, and the ionic strength of the carrier electrolyte are important parameters in optimization of nanoparticle separations. CZE is characterized by a high efficiency. However, in particle separations, band broadening is often observed because of particle electrophoretic heterogeneity and adsorption on capillary walls. To prevent particle–wall interactions, different variants of capillary modification are used. This technique is suitable for analysis of precious samples with limited availability because the sample volume required for measurements can be as small as several nanoliters. On the other hand, the small sample volume makes collection of fractions more difficult. Besides, the volumes collected may be too small for application of off-line detection techniques.

There are a variety of examples of successfully applying CZE to the separation and characterization of particles [97, 105–112]. Separation of polymers (polystyrene, polyaniline, etc.) and 0.6–1.3-nm single-walled carbon nanotubes [98] was reported. Size separations of metal nanoparticles such as gold (5–60 nm), gold clusters protected by citrate, SDS, and monolayers of *N*-acetyl-L-cysteine [113], silver nanoparticles (17–50 nm), and Au/Ag core–shell nanoparticles (24–90 nm) were performed. The correlation between electrophoretic mobility and the size of the nanoparticles is dependent on their zeta potential. Calculations by Wiersema et al. [114] showed that the relaxation effect has a strong impact on the resulting value of the electrophoretic mobility for nanoparticles with a high zeta potential. As a result, regions appear in which the electrophoretic mobility is negatively or positively correlated to the particle size. For example, addition of the surfactant SDS to the carrier electrolyte leads to an increase in the zeta potential of gold nanoparticles, resulting in an inversion of their migration order [97]. Combining CZE with *diode-array detection* offers the possibility to characterize nanoparticles not only on the basis of their electrokinetic properties but also according to their optic properties which depend on particle size and shape. Shape-based characterization of gold and silver nanoparticles was performed. Silver nanospheres and nanorods were distinguished in electrophoretic zones [115]. Reversed electrode polarity stacking mode can be applied to the online preconcentration of gold and Au/Ag particles [116, 117]. Enrichment efficiencies up to 260-fold were achieved.

The potential of separations of nanoparticle mixtures owing to differences in their size and chemical composition was also demonstrated for silica (5–500 nm) and different metal oxides such as γ -Al₂O₃, α -Al₂O₃, TiO₂, Fe₂O₃, Fe₃O₄, and UO₂ containing gold and polystyrene nanoparticles [118–121]. γ -Fe₂O₃ (magnetite, 6–10 nm) and ultrasmall (about 1 nm) carboxylate-functionalized silica

nanoparticles with different numbers of carboxylate linkers were separated by CZE and detected *using laser-induced fluorescence* (LIF) [122]. A correlation between the electrophoretic mobility of aminated magnetite/silica core-shell particles and the density of amino groups at their surface was established [123]. It was hence shown that CZE can be regarded as a fast and reliable method for the determination of the effective surface charge density of nanoparticles.

Recent publications [124, 125] have dealt with the separation of quantum dots—Cd/Se, Cd/Te, CdTe/CdS, CdSe/ZnS, CdSe/ZnS/SiO₂—using a polymer additive as the sieving medium. A correlation between the electrophoretic mobility and the size of the quantum dots was evaluated.

A commercial CZE system equipped with a LIF detector allows the separation and detection of individual particles if high separation efficiency and detection sensitivity are achieved. Besides, the spatial resolution, the data rate, and the rise time of the detector should be suitable for data acquisition with the device. Individual sulfated polystyrene particles (200–1,000 nm) were determined with a post-column LIF detector [126]. This equipment was also used for automated analysis of individual micrometer-sized particles [127]. The method developed may be extended to the size separation of nanoparticles.

The isoelectric point of nanoparticles has been shown to be size-dependent. This allows one to obtain fractions with a narrow size distribution using *isoelectric focusing electrophoresis* (IEF). Gold and silver nanoparticles (2.2–13 nm) coated with 4-carboxythiophenol were separated by IEF in a microcolumn [128]. The dependence of the isoelectric point on the particle size was ascribed to a dependence of the pK_a of carboxylic groups on the curvature of the particle surface. IEF in a polyacrylamide pH gradient gel was used for the size separation of gold nanoparticles (1.7–4.9 nm) protected by mercaptosuccinic acid [129].

Isotachopheresis (ITP) is a powerful separation technique employing a discontinuous electric field to create sharp boundaries between the sample constituents. In ITP, the sample is injected between a fast leading electrolyte (containing a co-ion with a higher electrophoretic mobility) and a slow terminating electrolyte (containing a co-ion with a lower electrophoretic mobility). After a voltage has been applied, a low electric field strength is created in the leading electrolyte and a high electric field strength is created in the terminating electrolyte. In the first stage, the sample constituents migrate at different velocities and start to separate from each other. The electric conductivity within a sample zone is proportional to the electrophoretic mobility of the particles separated. Size separations of gold nanoparticles (1–9 nm) protected with 11-mercaptoundecanoic acid, CdTe, and

CdSe/ZnS nanocomposites were performed [130, 131]. Although colloidal particles showed an electrophoretic heterogeneity, they can form correct isotachopheretic zones with sharp moving boundaries. ITP can be used as an absolute analytical method that enables the determination of the amount of particles in the injected sample volume without the requirement of calibration. It has also been shown [131] that ITP allows one to measure directly the effective charge of colloidal particles if solutions with known particle concentration can be prepared.

Dielectrophoresis (DEP) is an electrophoretic technique that was introduced in the 1950s. Recently, DEP has been revived owing to its potential in the manipulation of microparticles, nanoparticles, and cells. DEP occurs when a force is exerted on a dielectric particle placed in a nonuniform electric field. The time-averaged dielectrophoretic force acting on a particle depends on the effective polarizability, the particle volume, and the gradient of the energy density of the electric field [132]. The magnitude and direction of the dielectrophoretic force acting on a particle depends on its dielectric properties. Therefore, heterogeneous particle mixtures can be separated [133]. DEP can be enhanced by using multiple frequencies, and then DEP is known as *multiple-frequency DEP*.

The utilization of the difference between dielectrophoretic forces exerted on different particles in nonuniform electric fields is known as dielectrophoretic separation. The exploitation of dielectrophoretic forces has been classified into two groups: dielectrophoretic migration and dielectrophoretic retention. Dielectrophoretic migration uses dielectrophoretic forces that exert forces of opposite sign on different particle types to attract some of the particles and repel the others. Dielectrophoretic retention uses the balance between dielectrophoretic and fluid-flow forces. Particles experiencing repulsive and weak attractive dielectrophoretic forces are eluted by fluid flow, whereas particles experiencing strong attractive dielectrophoretic forces are trapped at electrode edges because of flow drag. This technique has found numerous applications in biotechnology and biomedical applications, including characterization of bioparticles [134]. The applications in nanotechnology were reviewed by Hughes [135] and Kadaksham et al. [136]. Recently, size separations of 0.8–1.4-nm single-walled carbon nanotubes, 250–12,000-nm polystyrene, 93–557-nm latex, and 100-nm silicon nanoparticles were reported [111]. DEP is also considered to be an effective fractionation method for metallic and semiconducting nanotubes owing to the very large differences in their polarizability [137]. DEP can be used for sorting single-walled carbon nanotubes according to their dielectric constant. In general, DEP is a good complementary technique to other techniques currently used for particle separations. It also enables small-scale preparative separation of nanoparticles to be performed.

Dielectrophoretic FFF combines the features of FFF and electrophoretic techniques. Particles are injected into a carrier flow that passes through the separation chamber, with an external separating force (a dielectrophoretic force) being applied perpendicular to the flow. By means of different factors, such as diffusion and steric, hydrodynamic, dielectric, and other effects, or a combination thereof, particles (less than 1 μm in diameter) with different dielectric or diffusive properties attain different positions away from the chamber wall, which, in turn, exhibit different characteristic concentration profiles. Particles that move further away from the wall reach higher positions in the parabolic velocity profile of the liquid flowing through the chamber and will be eluted from the chamber at a faster rate. Peng et al. [138] reported a dielectrophoretic FFF procedure for the separation of semiconducting single-walled carbon nanotubes.

Some other electrophoretic methods were also applied to the separation of nanoparticles. Cd/Te nanoparticles [139] were fractionated by *free flow electrophoresis*. Metallic or semiconducting single-walled carbon nanotubes [140] were fractionated by *free solution electrophoresis*. The reversed electrode polarity stacking mode of *micellar electrokinetic chromatography* was used for the online preconcentration and separation of gold nanoparticles (5 and 40 nm) [141].

Microfluidic techniques

Microfluidic systems employ quite different separation principles and often provide a basis for the fabrication of *lab-on-chip* devices. A new microfluidic approach for charge-based [142] and size-based [143] particle separation has been proposed using combined hydrodynamic and electrokinetic effects. A recirculating flow pattern is employed, generated through application of bidirectional flow in a narrow glass microchannel. The bidirectional flow in turn is a result of opposing pressure-driven and electroosmotic flows. Trapping and preconcentration of charged particles is observed in the recirculating flow under conditions where the average net velocity of the particles themselves approaches zero. This phenomenon is termed “*flow-induced electrokinetic trapping*.” As a result, charge-based separations of polystyrene beads with different zeta potentials and size-based separation of polymer microparticles with the same zeta potential were performed with a resolution comparable to that of baseline separation in chromatography. One of the main advantages of this method is that it can be applied in short (a few millimeters) channel geometries.

On-chip planar HDC was suggested [144]; it is suitable for size characterization of synthetic polymers, biopolymers, and particles. Possible advantages of on-chip HDC over conventional techniques, such as GPC and FFF, are

fast analysis, high efficiency, reduced solvent consumption, and easy temperature control. In a planar configuration realized in fused silica, size separations of polystyrene nanoparticles were performed.

Spiral microchannels employing Dean flows and differential migration can be applied to the separation of microparticles (1–20 μm) [145, 146]. A new microfluidic method, *multiorifice flow fractionation*, was recently designed for continuous size-based separation of spherical microparticles [147]. This method utilizes inertial lift force and momentum-change-induced inertial force generated in a series of contraction/expansion microchannels. A multi-layered polydimethylsiloxane microfluidic device was proposed for the size-based separation of heterogeneous microbial samples [148]. The recent developments in microfluidic technology for cell and particle separation in continuous-flow-based systems were critically reviewed by Lenshof and Laurell [6].

Other methods

A number of other techniques have also been used for the fractionation of macromolecules and particles. Critical analysis of all these techniques is beyond the scope of this review; however, some of them should be mentioned. *Centrifugation* and *ultracentrifugation* can be applied to the separation of silica [149] modified gold [150] and other [151, 152] nanoparticles using a centrifugal force field. Separation and concomitant purification of nanoparticles by ultracentrifugation in a nonhydroxylic *organic density gradient* has been demonstrated by separating several typical colloidal nanoparticles, including gold, silver, and CdSe. Successful separation of gold nanowires from their spherical counterparts showed that colloidal particles can be separated not only by size but also by morphology [153].

A new strategy for separation of microparticles and nanoparticles with different sizes and densities was suggested using the *tensions at liquid interfaces* [154]. Size-based separations of Cu_2O nanoparticles were achieved with a water/*n*-pentanol interface. Size-dependent reversible *salt-induced aggregation* of particles in aqueous solutions combined with nucleotide-mediated stabilization was applied to gold nanoparticles [155]. The use of the pressure-tunable physicochemical properties of *CO₂-expanded liquids* provides a basis for a rapid, precise, and environmentally sustainable size-selective fractionation of ligand-stabilized nanoparticles at an application scale by varying the CO_2 pressure [156]. A simple and precise size separation of polystyrene microparticles is possible using a *nanogap method* based on a narrow gap between a cylindrical lens and a flat glass wall, in which the distance of the gap is precisely determined by the diffraction moiré pattern of irradiated light [157]. Micron and submicron

biological particles can be sorted by *hydrophoresis* using *anisotropic microfluidic obstacles* [8]. The separation mechanism is based on the hydrodynamics of rotational flows induced by anisotropic obstacles.

Trapping, transportation, and separation of small particles, e.g., plant seeds, in a liquid is possible using an *acoustic needle* which is driven by a sandwich-type ultrasonic transducer [158]. The trapped particles can be transported in water by moving the needle, which can separate different particles by the difference in their sizes and densities. Acoustic radiation forces can be used not only for the fractionation of total amounts of micrometer-sized suspended particles but for single particle manipulation as well. This has been demonstrated by manipulation of copolymer beads and cells using a *microgripper* [159].

Characterization of nanoparticles and microparticles

Nanoparticles and microparticles can differ from each other in chemical composition, size, shape, heterogeneity, surface modification, optical properties, magnetic susceptibility, etc. In terms of shape, one can distinguish spheres, rods, plates, tubes, wires, etc. All these parameters determine the physicochemical properties of particles.

As mentioned already, FFF, chromatographic, and electrokinetic methods with conventional UV–vis spectrophotometric detection can be used not only for fractionation but also for sizing, characterization, and investigation of nanoparticles and microparticles. For instance, the mean diameters of industrial carbon blacks were estimated by SdFFF and FIFFF [33]. The results were correlated with the data obtained by *photon correlation spectroscopy*, also known as *dynamic light scattering* (DLS). The mean diameters measured by two different FFF subtechniques and photon correlation spectroscopy were found to be in good agreement within 20%. FIFFF in a hyperlayer operation mode was proven to be an appropriate technique for the characterization of porous hybrid silica and silica chromatographic support particles ranging approximately from 1 to 15 μm [160]. The comparison of the FFF results and the *scanning electron microscopy* data further confirmed that good accuracy can be achieved by the flow/hyperlayer FFF method for particle size analysis of hybrid silica and silica particles.

Interactions between nanoparticles and molecules occurring in solutions can be studied by electrophoretic techniques. CZE was used to characterize noncovalent binding of a stabilizer (citrate) to the gold nanoparticle surface [100] and to study CdSe quantum dot surface–ligand interactions [125]. Agarose GE was applied to the determination of size [102] and stoichiometry [97, 161] of nanoparticle–

biomolecule conjugates. Electrophoretic techniques also provide a basis for particle surface characterization (zeta potential, isoelectric point) and suspension stability studies (kinetics of aggregation and disaggregation, adsorbate–particle interactions in suspensions). A series of dielectrophoretic FFF applications to the analysis of dielectric, density, and deformability characteristics of cells and particles have been reviewed recently [162].

Electron microscopy (scanning or transmission) is a unique technique that allows one to visualize the nanoparticles and microparticles under study and simultaneously determine their elemental composition if an energy-dispersive device is available. However, electron microscopy requires dried samples for investigation. The initial equilibria occurring in a liquid medium may shift significantly, particles can aggregate owing to drying, and their surface properties may change.

For sizing and characterization of particles in liquid media, the following techniques can be used:

- *Gravitational photosedimentation*. The settling velocity of a particle suspended in water depends on the nature of the acceleration field (e.g., gravitational or centrifugal), namely, the particle mass and friction coefficient. If particle concentrations are relatively low (ideally less than 0.5%) and the settling is not “hindered” by particle–particle interactions, it is possible to convert settling velocity measurements into estimates of particle size or volume. The size range that can be measured using standard gravitational sedimentation instruments is typically from 10 to 300 μm , although the lower boundary can be decreased [4].
- *Centrifugal photosedimentation*. If settling experiments are carried out in a centrifuge, where the gravitational field is replaced by the centrifugal acceleration, it becomes possible to sense the settling rate of particles starting from 0.01 μm . Centrifugal photosedimentation allows one to detect particles that are either higher or lower in density than the fluid in which they are suspended. Particles that are higher in density than the fluid settle at the point furthest from the center of rotation (bottom), whereas particles lower in density than the fluid float toward the point closest to the center of rotation (top). The method is time-consuming owing to the overhead associated with stopping, emptying, and cleaning the centrifuge after each sample has been processed. Besides, the particle size distribution may change significantly during the centrifugation process [4]. In addition, density inhomogeneity of particles (e.g., core–shell nanocomposites) may affect the results obtained.

The characterization and sizing using sedimentation techniques are based on the separation of particles. The

techniques described below allow one to characterize particles without their separation:

- *Blockage or light obscuration techniques.* The main principle of this approach is the following: individual particles are forced to flow through an illuminated sensing zone, momentarily casting a shadow on a photodiode (detector). When a series of sensors are employed, the particle size range that can be resolved is from about 1 μm to 1 mm. The size range for a single light obscuration sensor is typically from 1 to 200 μm , with sample flow rates between 10 and 100 mL/min. The method is frequently used for online monitoring in potable water treatment [4].
- *Laser light scattering technique: diffraction instruments.* Diffraction theory can be applied in the case where the suspended particles are much larger than the wavelength of incident light. Particles migrate into the laser beam and scatter light, which is focused by a lens onto the detector. Lenses of different focal lengths can be employed to focus on different portions of the particle size spectrum; typical lenses and detector combinations can resolve particle sizes in the range from 1 to 600 μm . It is even possible to extend the range to over 2 mm. [4].
- *Laser light scattering technique: dynamic (time-dependent).* The method is based on the measurement of Brownian motion and the correlation of it to the size of particles. Smaller particles move faster than larger ones and the intensity of their speckle pattern fluctuates faster. By illuminating the particles with a laser and analyzing the intensity fluctuations in the scattered light, one can determine the particle size distribution of the suspended particles. The equivalent sphere diameter (hydrodynamic diameter) may be larger than the diameter of a particle measured by electron microscopy, because a layer of solvent molecules may be included in the hydrodynamic diameter. For polydisperse samples, the interpretation of DLS data is difficult [4]. Nevertheless, DLS is probably the most favored detection method for colloids and solid particles in the size range from 1 nm to 5 μm . The method is most frequently used for the off-line and online detection and characterization of particles after their separation in FFF systems.
- *Laser light scattering technique: static (time-averaged).* When the test sample being analyzed contains macromolecules, this method is useful for determining the molecular weight and the radius of gyration (a size parameter in the shape factor) from the integrated or time-averaged intensity of scattered light. These features can be employed to characterize natural colloidal and particulate matter. However, time-averaged intensity measurements are often plagued by the presence of very large particles in the suspension. The presence of very large particles leads to a systematic increase in scattering intensity. The only way to handle “oversized” particles is to remove them during the sample preparation procedure [4]. Besides, one should be cautious to apply static light scattering to small particles, say, smaller than 10 μm , depending on the refractive indices of the particles and the medium as well as on the instrumentation used. Since the particle size distribution is determined by the splitting of detector signals from all existing particles, additional populations could be created by the analysis procedure, and particle size distributions could be seriously distorted [160].
- *Laser Doppler velocimetry.* This is a well-established technique for measuring the velocity of particles moving through a fluid on the basis of the Doppler effect. The rate of intensity fluctuations in scattered light is proportional to the particle velocity. Using a combination of electrophoresis and Laser Doppler velocimetry allows one to measure a particle velocity in a liquid when an electric field is applied. As a result, the electrophoretic mobility and the zeta potential of particles can be calculated. The *Zetasizer Nano* system manufactured by Malvern Instruments, UK, provides the ability to measure three characteristics of particles in a liquid medium – particle size, zeta potential, and molecular weight – over a wide concentration range. For particles in the range from 5 nm to 10 μm , it is possible to determine the zeta potential by using a combination of electrophoresis and phase analysis light scattering. Zetasizer systems can be used to measure the particle sizes in suspensions over a wide size range without an equilibrium shift. However, only suspensions containing particles of the same size or particles of sizes differing substantially can be analyzed successfully.
- *Laser-induced fluorescence (LIF)* is a spectroscopic method used for characterizing molecules, colloids, and solid nanoparticles. The wavelength-resolved fluorescent emission can provide a tool for the assessment of nanoparticle functionalization [122]. Fluorescence spectra provide important information on the structure of single-walled carbon nanotubes [163]. LIF characterization (information on chain dynamics) of polymeric coated superparamagnetic nanoparticles can be also achieved [164].
- The *electrical resistive pulse technique* is based on the difference in the resistance (or conductivity) of a particle suspended in an electric field and that of the suspending fluid. If a nonconducting particle suspended in a weak electrolyte (e.g., 1–4% aqueous solution of NaCl) is placed in a small aperture, an

increase in resistance across the orifice relative to that of the medium alone is produced. The magnitude of this increase in resistance is proportional to the particle volume. The method is applicable to particles in the size range from 1 μm to 1 mm. It should be noted, however, that the conductive carrier fluid may promote particle coagulation and therefore affect the measurement process [4].

- The *electrical sensing zone method* (often called the *Coulter method*) is currently one of the main technologies used by chromatographic material manufacturers in particle size analyses of chromatographic support particles in the micron size range. In an electrical sensing zone system, an aperture is placed between two electrodes and a suspension flows through the aperture. As a solid particle passes through the aperture, it displaces its own volume of conducting liquid, momentarily increasing the impedance of the aperture. The change in the impedance is converted to a voltage pulse. The amplitude of the pulse is proportional to the volume of the electrolyte displaced by the solid particle and the number of pulses is the number of particles measured. For porous particles, the electrolyte can wet pores and particle diameters are underestimated [160].
- *Electrokinetic sonic amplitude measurements (acousto-sizer systems)*. An alternating electric field applied to a suspension of charged particles causes particle movement back and forth. Particle oscillations generate sound waves if there is a density difference between the particles and the dispersing medium. The acoustic waves are detected and the particle dynamic mobility is calculated using the signal intensity. The method allows one to determine particle size (in the range 0.02–10 μm), zeta potential, and isoelectric point in dispersions with particle concentrations of 1.0–40% v/v. The change in dynamic mobility upon addition of a polymer to the particle dispersion reflects the formation of polymer layers on the particle surface [165]. This can be used for assessment of suspension stabilization.

The basic principles, merits, and limitations of some of the methods described above are discussed in detail in a book by Grant et al. [4]. Some other methods should also be mentioned here. The *Taylor dispersion method* is related to the development of velocity profiles in a fluid plug flowed through a tube. The simultaneous action of convection and diffusion on species driven hydrodynamically through a capillary tube leads to a plug broadening. Taylor dispersion may be used for calculation of diffusion coefficients and estimation of hydrodynamic radii of nanoparticles [166]. This method is a powerful tool for the sizing of proteins and one can also perceive it as a useful analytical approach for characterization of relatively mono-

disperse suspensions of nanoparticles. *Nanoparticle tracking analysis* can successfully be applied to aquatic environmental samples [167]. The method has certain advantages, such as minimum perturbation of the samples, high sensitivity in terms of particle concentration, and provision of number-based size distributions. The number concentrations obtained for natural nanoparticles correlate well with those obtained from conventional turbidity measurements. It should be noted that the list of techniques presented here is not exhaustive.

With regard to the elemental analysis of colloidal and particulate matter in suspensions, spectrometric methods such as ICP-MS and ICP-AES are mainly applied.

“Hyphenated” techniques

By definition, chromatography, FFF, or electrokinetic techniques employing online UV–vis spectrometric or other conventional detectors are attributed to “hyphenated” ones. In this review, however, special emphasis is given to the novel approaches utilizing instrumental analytical methods such as ICP-MS, static light scattering, DLS, etc. The “hyphenated” techniques described below are grouped according to the separation methods:

- *Field-flow fractionation (FFF)*. AF4 coupled with static light scattering has been successfully applied to the characterization of colloidal dispersions undergoing aggregation. An example is conventional polystyrene latex and a dispersion of elastomeric colloidal particles made of a polymer with a low glass-transition temperature [168]. AF4 coupled online with small-angle X-ray scattering (SAXS) and DLS can be a powerful tool in polymer analysis. A mixture of six polystyrene sulfonates with molar masses in the range from 6.5×10^3 to 1.0×10^6 g/mol was used as a model system for polyelectrolytes in aqueous solutions with a broad molar mass distribution. Detailed information for all polymer fractions is available on (1) the radii of gyration, which were determined from the SAXS data interpretation in terms of the Debye model (Gaussian chains) and (2) the diffusion coefficients (from DLS). An AF4-SAXS-DLS combination may even be recommended as a possible new reference method for the detailed analysis of complex polymer mixtures [169].

AF4 coupled with multiangle laser light scattering (MALLS) detection was used for the investigation [58] and quantitative characterization [59] of viruslike particles. The composition of samples obtained upon complexation of DNA with chitosan was analyzed by AF4 with online UV–vis, MALLS, and DLS detectors. AF4 combined with DLS allowed the characterization of small particles that were not

Table 1 Some examples of choosing appropriate methods for separation, detection, and analysis of particles

Sample	Task	Methods
Environmental macromolecules, colloidal and particulate matter (soils, leachates, aquatic samples)	Separation, size characterization, and study of element associations to different fractions	Asymmetrical flow FFF coupled with static multiangle light scattering and ICP-MS [171, 172]
Silica and polystyrene latex nanoparticles	Size separation	Gel permeation chromatography with sequential detection by dynamic light scattering [176, 177]
Solid and hollow polystyrene latexes	Size and shape characterization	A quadruple-detector hydrodynamic chromatography method utilizing static multiangle light scattering, dynamic light scattering, differential viscometry, and differential refractometry [178]
Gold nanoparticles	Size separation as well as separation according to the shape (spheres, plates, rods)	Gel electrophoresis with subsequent detection and visualization of particles by electron microscopy [99]
Gold and silver nanoparticles (nanorods)	Size and shape separation, chemical characterization	Combining capillary zone electrophoresis with diode-array detection [115, 116]

FFF field-flow fractionation, ICP-MS inductively coupled plasma mass spectrometry

detected by conventional batch-mode DLS. The AF4 analysis can be beneficial in the field of gene therapy because it readily provides, in a single measurement, three important physicochemical parameters of the complexes: the amount of unbound polycation, the hydrodynamic size of the complexes, and their size distribution [170].

The challenging task of characterizing polydisperse polymer mixtures containing ultrahigh molecular weight polymers (polyvinyl acetate) and ultrahigh molecular weight microgel components in organic solvents is addressed with TFFF and MALLS–differential refractive index detection [38].

Metal associations to different environmental microparticles, nanocolloids, and macromolecules can be studied using AF4 coupled with ICP-MS. A methodological approach based on the size characterization of environmental microparticles (size larger than 1 μm), nanocolloids

(1 μm to 15 nm), and macromolecules (smaller than 1,000 kDa) by AF4 taking advantage of both normal and steric elution modes was proposed [171]. The procedure was optimized to minimize the potential alteration of the size distribution and metal associations of the species characterized. The contribution of three differentiated fractions to the mobilization of metals in compost leachates was assessed.

AF4 coupled online with a multidetection system comprising a UV detector, MALLS, and ICP-MS has been proven to be a powerful “hyphenated” technique for characterizing colloidal transport of uranium in soil [172]. Moreover, with use of AF4, the different colloidal fractions were collected and characterized by a total organic carbon analyzer. Thus, it was possible to determine the nature (organic or inorganic colloids), molar mass, size (gyration and hydrodynamic radii), and quantitative uranium distribution

Table 2 Characteristics of the most representative methods for fractionation of nanoparticles and microparticles

Methods	Separation according to	Working size range	Sample handling
Membrane filtration	Size	10 nm to 10 μm	Up to 1 kg of particulate matter (for continuous fractionation)
Flow FFF	Size, density	1 nm to 50 μm	Up to 1 mg
Thermal FFF	Size, chemical composition	50 nm to 10 μm	Up to 1 mg
Sedimentation FFF	Size, density	50 nm to 50 μm	Up to 1 mg
Coiled-tube FFF	Size, density	100 nm to 200 μm	Up to 500 mg
SPLITT separation	Size, density	1–50 μm	Continuous fractionation
Gel permeation chromatography	Size, shape	1–100 nm	Up to 100 μL
Hydrodynamic chromatography	Size	10 nm to 10 μm	1–10 μL
Capillary zone electrophoresis	Size, shape, zeta potential	1 nm to 1 μm	1–10 nL
Capillary isotachopheresis	Size, concentration, effective charge	1 nm to 1 μm	0.1–100 μL

SPLITT split-flow thin-cell

over the whole colloidal phase. It was found that humic substances are mainly responsible for uranium transport in subsurface conditions, whereas nanoparticles are most important under depth conditions [172]. Combining AF4 with MALLS and ICP-MS was also applied to the characterization of nanoclay used in biopolymer nanocomposites [173].

- *Liquid chromatography (LC)*. Online coupling of LC with ICP-MS was used for nanoparticle characterization (i.e., size, composition, presence of impurities, etc.) [174] and size separation [100]. Separation and quantitation of fullerenes was performed by LC coupled with electrospray ionization mass spectrometry [175]
- *Gel permeation chromatography (GPC)*. A new method has been presented [176] based on the size separation of silica colloids by GPC and their sequential determination by DLS. For nanoparticles with diameters below 100 nm, use of a microchannel GPC chip was suggested [177]. In this case, standard polystyrene latex nanoparticles with diameters of 20 and 100 nm were studied. Good agreement between the simulation and measurement results showed the usefulness of this method.
- *Hydrodynamic chromatography (HDC)*. A quadrupole-detector HDC method utilizing static multiangle light scattering, DLS, differential viscometry, and differential refractometry has been introduced and applied to characterizing a series of solid and hollow polystyrene latexes with diameters in the approximate range of 40–400 nm [178]. It became possible to determine a multiplicity of size parameters and their polydispersity and to monitor the size of particles across the elution profile of each sample. With use of self-similarity scaling relationships between the molar mass and the various particle radii, the shape of the latexes and the shape constancy as a function of particle size were ascertained. Solid and hollow polystyrene latex samples were differentiated.

A “hyphenated” method has been developed and validated which utilized the ability of HDC to separate particles in a wide size range combined with the multielement selectivity of ICP-MS [179]. The method was applied to the analysis of metal-based nanoparticles (TiO_2 , SiO_2 , Al_2O_3 , Fe_2O_3 , and Ag) in environmental samples. The online combination is characterized by a short analysis time (less than 10 min), an extended sizing range (5–300 nm), and limited sample pretreatment.

- *Gel electrophoresis (GE)* coupled online with ICP-MS was also tested and applied to the size characterization of gold nanoparticle standards [100].

In general, further development of “hyphenated” techniques is promising and may open a new door to the separation, characterization, and analysis of nanoparticles and microparticles of different type and origin.

Conclusions

It can be concluded that the selection of appropriate fractionation, detection, and analytical techniques is dependent on the research tasks as well as on particle size, density, shape, and surface properties. A few representative examples are given in Table 1.

Besides, the development of combined fractionation methods based on the sequential separation of complex mixtures of particulate species using various approaches looks promising. Combinations should be worked out taking into account that:

- The efficiency of separation of particles for the subsequent technique should be higher than for the preceding one.
- The separation principles of the methods should be different (separation according to size, shape, density, charge, etc.).
- The initial separation should be carried out according to the size that makes it possible to create an even scale for various samples.

The brief summary of the most representative fractionation methods given in Table 2 may be useful for choosing appropriate techniques.

Acknowledgments The authors are grateful to the Russian Foundation of Basic Research for financial support.

References

1. Buffle J, van Leeuwen HP (1993) Environmental particles. Lewis, Boca Raton
2. Gimbert LJ, Andrew KN, Haygarth PM, Worsfold PJ (2003) Trends Anal Chem 22:615–633
3. Tang ZY, Wu LH, Luo YM, Christie P (2009) Environ Geochem Health 31:1–10
4. Grant SB, Rekhi NV, Pise NR, Reeves RL, Matsumoto M, Wistrom A, Moussa L, Bay S, Kayhanian M (2003) A review of the contaminants and toxicity associated with particles in stormwater runoff. California Department of Transportation, Los Angeles
5. Selbig WR, Bowman G (2007) J Environ Qual 36:226–232
6. Lenshof A, Laurell T (2010) Chem Soc Rev 39:1203–1217
7. Helmbrecht C, Niessner R, Haisch C (2007) Anal Chem 79:7097–7103
8. Choi S, Song S, Choi C, Park JK (2009) Anal Chem 81:50–55
9. Spivakov BYa, Shkinev VM (2005) In: Encyclopedia of analytical science, vol 5. Elsevier, London, pp 524–530
10. Sigg L, Black F, Buffle J, Cao J, Cleven R, Davison W, Galceran J, Zhang H (2006) Environ Sci Technol 40:1934–1941

11. Gimpel J, Zhang H, Davison W, Edwards AC (2003) *Environ Sci Technol* 37:138–146
12. Sweeney SF, Woehrl GH, Hutchison JE (2006) *J Am Chem Soc* 128:3190–3197
13. Trefry JC, Monahan JL, Weaver KM, Meyerhoefer AJ, Markopolous MM, Arnold ZS, Wooley DP, Pavel IE (2010) *J Am Chem Soc* 132:10970–10972
14. Jermann D, Pronk W, Boller M (2008) *Environ Sci Technol* 42:9129–9136
15. Hwang KJ, Liao CY, Tung KL (2008) *Desalination* 234:16–23
16. Trofimov DA, Shkinev VM, Spivakov BYa, Schué F (2009) *J Membr Sci* 326:265–269
17. Rahimpour A, Madaeni SS, Taheri AH, Mansourpanah Y (2008) *J Membr Sci* 313:158–169
18. Frederic JD, Leanne M, Jamie RL (2005) *Talanta* 67:144–154
19. Itoh A, Nagasawa T, Zhu YB, Lee KH, Fujimori E, Haraguchi H (2004) *Anal Sci* 20:29–36
20. Ziyang L, Youcai Z (2007) *J Hazard Mater* 147:257–264
21. Shkinev VM (2001) *Membr Technol* 134:8–10
22. Buykx SEJ, Marc AGT, Van Den Hoop, Rob FM, Cleven J, Buffle J, Wilkinson KJ (2000) *Int J Environ Anal Chem* 77:75–93
23. Shkinev VM, Dzherayan TG, Karandashev VK, Arakchaa KD, BYa S (2000) *J Anal Chem* 55:135–141
24. Stepanets OV, Ligaev AN, Borisov AP, Travkina AV, Shkinev VM, Danilova TV, Miroshnikov AYu, Migunov VI (2009) *Geochem Int* 47:657–672
25. Giddings JC (1966) *Sep Sci* 1:123–125
26. Schimpf M, Caldwell KD, Giddings JC (2000) *Field-flow fractionation handbook*. Wiley, New York
27. Kowalkowski T, Buszewski B, Cantado C, Dondi F (2006) *Crit Rev Anal Chem* 36:129–135
28. Chianéa T, Assidjo NE, Cardot PJP (2000) *Talanta* 51:835–847
29. Giddings JC (1978) *Sep Sci Technol* 13:241–254
30. Caldwell KD, Nguyen TT, Myers MN, Giddings JC (1979) *Sep Sci Technol* 14:935–946
31. Giddings JC (1997) *Anal Chem* 69:552–557
32. Dubascoux S, Von Der Kammer F, Le Hecho I, Potin Gautier M, Lespes G (2008) *J Chromatogr A* 1206:60–165
33. Park YH, Kim WS, Lee DW (2003) *Anal Bioanal Chem* 375:489–495
34. Deering CE, Tadjiki S, Assemi S, Miller JD, Yost GS, Veranth JM (2008) *Part Fibre Toxicol*. doi:10.1186/1743-8977-5-18
35. Tadjiki S, Assemi S, Deering CE, Veranth JM, Miller JD (2009) *J Nanopart Res* 11:981–988
36. Kim ST, Kang DY, Lee SH, Kim WS, Lee JT, Cho HS, Kim SH (2007) *J Liq Chromatogr Relat Technol* 30:2533–2544
37. Contado C, Argazzi R (2009) *J Chromatogr A* 1216:9088–9098
38. Lee D, Williams SKR (2010) *J Chromatogr A* 1217:1667–1673
39. Messaud FA, Sanderson RD, Runyon JR, Otte T, Pasch H, Williams SKR (2009) *Prog Polym Sci* 34:351–368
40. Shiundu PM, Williams SKR (2004) *ACS Symp Ser* 881:185–198
41. Janča J, Berneron JF, Boutin R (2003) *J Colloid Interface Sci* 260:317–323
42. Edwards TL, Gale BK, Frazier AB (2002) *Anal Chem* 74:1211–1216
43. Janča J, Ananieva IA, Menshikova AY, Evseeva TG (2004) *J Chromatogr B* 800:33–40
44. Janča J, Stejskal J (2009) *J Chromatogr A* 1216:9071–9080
45. Janča J, Halabalova V, Polasek V, Vasina M, Menshikova AYu (2010) *Int J Polym Anal Charact* 15:191–197
46. Giddings JC, Yang FJ, Myers MN (1976) *Science* 193:1244–1245
47. Vaillancourt RD, Balch WM (2000) *Limnol Oceanogr* 45:485–492
48. Baalousha M, Kammer FVD, Motelica-Heino M, Hilal HS, Le Coustumer PJ (2006) *J Chromatogr A* 1104:272–281
49. Song JH, Kim WS, Lee DW (2003) *J Liq Chromatogr Relat Technol* 26:3003–3035
50. Ahn JY, Kim KH, Lee JY, Williams PS, Moon MH (2010) *J Chromatogr A* 1217:3876–3880
51. Moon MH, Kang DJ, Jung JH, Kim JM (2004) *J Sep Sci* 27:710–717
52. Yohannes G, Wiedmer SK, Jussila M, Riekkola ML (2005) *Chromatographia* 61:359–364
53. Stolpe B, Hasselov M (2007) *Geochim Cosmochim Acta* 71:3292–3301
54. Alasonati E, Benincasa MA, Slaveykova VI (2007) *J Sep Sci* 30:2332–2340
55. Thunemann AF, Knappe P, Bienert R, Weidner S (2009) *Anal Methods* 1:177–182
56. Zillies JC, Zwiroek K, Winter G, Coester C (2007) *Anal Chem* 79:4574–4580
57. Isaacson CW, Bouchard D (2010) *J Chromatogr A* 1217:1506–1512
58. Lang R, Vogt L, Zurcher A, Winter G (2009) *LC GC N Am* 27:844–852
59. Pease LF, Lipin DI, Tsai DH, Zachariah MR, Lua LHL, Tarlov MJ, Middelberg APJ (2009) *Biotechnol Bioeng* 102:845–855
60. Capote FP, Luque de Castro MD (2007) *Anal Bioanal Chem* 387:249–257
61. Masudo T, Okada T (2001) *Anal Chem* 73:3467–3471
62. Mandralis ZI, Feke DL (1993) *Chem Eng Sci* 48:3897–3905
63. Masudo T, Okada T (2006) *Curr Anal Chem* 2:213–227
64. Fedotov PS, BYa S, Shkinev VM (2000) *Anal Sci* 16:535–536
65. Fedotov PS (2002) *J Liq Chromatogr Relat Technol* 25:2065–2078
66. Katasonova ON, Fedotov PS, BYa S, Philippov MN (2003) *J Anal Chem* 58:529–533
67. Fedotov PS, Kronrod VA, Katasonova ON (2005) *J Anal Chem* 60:349–356
68. Fedotov PS, Ermolin MN, Savonina EYu, Kronrod VA, BYa S (2010) *J Anal Chem* 65:1209–1214
69. Katasonova ON, Fedotov PS, Karandashev VK, BYa S (2005) *J Anal Chem* 60:765–773
70. Giddings JC (1985) *Sep Sci Technol* 20:749–768
71. De Momi A, Lead JR (2008) *Sci Total Environ* 405:317–323
72. Moon MH, Yang SG, Lee JY, Lee S (2005) *Anal Bioanal Chem* 381:1299–1304
73. Storey J, Douglas P, Ligrani P, Morten K (2009) *Sep Sci Technol* 44:1895–1922
74. Lee S, Cho SK, Yoon JW, Choi SH, Chun JH, Eum CH, Kwen H (2010) *J Liq Chromatogr Relat Technol* 33:27–36
75. Ratier C, Hoyos M (2010) *Anal Chem* 82:1318–1325
76. Lee S, Lee TW, Cho SK, Kim ST, Kang DY, Kwen H, Lee SK, Eum CH (2010) *Microchem J* 95:11–19
77. Asparnut Z, Yamada T, Lim LW, Takeuchi T (2008) *Anal Bioanal Chem* 391:353–359
78. Hradila J, Pisareva A, Babica M, Horak D (2007) *China Particuol* 5:162–168
79. Gautier C, Taras R, Gladiali S, Burgi T (2008) *Chirality* 20:486–493
80. Liu FK (2007) *Chromatographia* 66:791–796
81. Liu FK (2008) *Chromatographia* 68:81–87
82. Liu FK (2009) *Chromatographia* 70:7–13
83. Wei GT, Liu FK, Wang CRC (1999) *Anal Chem* 71:2085–2091
84. Arita T, Yoshimura T, Adschiri T (2010) *Nanoscale* 2:1467–1473
85. Al-Somali AM, Krueger KM, Falkner JC, Colvin VL (2004) *Anal Chem* 76:5903–5910
86. Small HJ (2004) *Colloid Interface Sci* 48:147–161
87. Takeuchi T, Siswoyo Aspanut Z, Lim LW (2009) *Anal Sci* 25:301–306
88. Thompson JW, Lieberman RA, Jorgenson JW (2009) *J Chromatogr A* 1216:7732–7738

89. Fischer CH, Giersig M (1994) *J Chromatogr A* 688:97–105
90. Umehara R, Harada M, Okada T (2009) *J Sep Sci* 32:472–478
91. Song Y, Jimenez V, McKinney C, Donkers R, Murray RW (2003) *Anal Chem* 75:5088–5096
92. Song Y, Heien MLAV, Jimenez V, Wightman RM, Murray RW (2004) *Anal Chem* 76:4911–4919
93. Zhang Y, Shuang SM, Dong C, Lo CK, Paa MC, Choi MMF (2009) *Anal Chem* 81:1676–1685
94. Liu FK, Chang YC (2010) *J Chromatogr A* 1217:1647–1653
95. Choi MMF, Douglas AD, Murray RW (2006) *Anal Chem* 78:2779–2785
96. Shen CW, Yu T (2009) *J Chromatogr A* 1216:5962–5967
97. Pyell U (2010) *Electrophoresis* 31:814–831
98. Hersam MC (2008) *Nat Nanotechnol* 3:387–394
99. Xu X, Caswell KK, Tucker E, Kabisatpathy S, Brodhacker KL, Scrivens WA (2007) *J Chromatogr A* 1167:35–41
100. Helfrich A, Brüchert W, Bettner J (2006) *J Anal At Spectrom* 21:431–434
101. Hanauer M, Pierrat S, Zins I, Lotz A, Sönnichsen C (2007) *Nano Lett* 7:2881–2885
102. Parak WJ, Pellegrino T, Micheel CM, Gerion D, Williams SC, Alivisatos AP (2003) *Nano Lett* 3:33–36
103. Song XT, Li L, Chan HF, Fang NH, Ren JC (2006) *Electrophoresis* 27:1341–1346
104. Vicente G, Colon LA (2008) *Anal Chem* 80:1988–1994
105. Radko SP, Chrambach A (2002) *Electrophoresis* 23:1957–1972
106. Hwang WM, Lee CY, Boo DW, Choi JG (2003) *Bull Korean Chem Soc* 24:684–686
107. Liu FK, Ko FH (2004) *Chem Lett* 33:902–903
108. Rodrigues MA, Armstrong DW (2004) *J Chromatogr B* 800:7–25
109. Cottet H, Simó C, Vayaboury W, Cifuentes A (2005) *J Chromatogr A* 1068:59–73
110. Kostal V, Arriaga EA (2008) *Electrophoresis* 29:2578–2586
111. Surugau N, Urban PL (2009) *J Sep Sci* 32:1889–1906
112. Klodzinska E, Buszewski B (2009) *Anal Chem* 81:8–15
113. Lo CK, Paa MC, Xiao D, Choi MMF (2008) *Electrophoresis* 29:2330–2339
114. Wiersema PH, Loeb AL, Overbeek JTG (1966) *J Colloid Interface Sci* 22:78–99
115. Liu FK, Ko FH, Huang PW, Wu CH, Chu TC (2005) *J Chromatogr A* 1062:139–145
116. Lin KH, Chu TC, Liu FK (2007) *J Chromatogr A* 1161:314–321
117. Liu FK (2007) *J Chromatogr A* 1167:231–235
118. Quang C, Petersen SL, Ducatte GR, Ballou NE (1996) *J Chromatogr A* 732:377–384
119. Petersen SL, Ballou NE (1999) *J Chromatogr A* 834:445–452
120. Vanífatova NG, Spivakov BYa, Mattusch J, Wennrich R (2003) *Talanta* 59:345–353
121. Vanífatova NG, Spivakov BYa, Mattusch J, Frank U, Wennrich R (2005) *Talanta* 66:605–610
122. Eckhoff DA, Stuart JN, Sutin JDB, Sweedler JV, Gratton E (2006) *J Chem Phys* 125:0811031–0811034
123. d'orlyé F, Varenne A, Georgelin T, Siaugue JM, Teste B, Descroix S, Gareil P (2009) *Electrophoresis* 30:2572–2582
124. Li YQ, Wang HQ, Wang JH, Guan LY, Liu BF, Zhao YD, Chen H (2009) *Anal Chim Acta* 647:219–225
125. Oszwaldowski S, Zawistowska K, Grigsby LK, Roberts KR (2010) *Cent Eur J Chem* 8:806–819
126. Duffy CF, McEathron FF, Arriaga EA (2002) *Electrophoresis* 23:2040–2047
127. Ahmadzadeh H, Dua R, Presley AD, Arriaga EA (2005) *J Chromatogr A* 1064:107–114
128. Gole AM, Sathivel C, Lachke A, Sastry M (1999) *J Chromatogr A* 848:485–490
129. Arnaud I, Abid JP, Roussel C, Girault HH (2005) *Chem Commun* 787–788
130. Bücking W, Nann T (2006) *IEE Proc Nanobiotechnol* 153:47–53
131. Pyell U, Bücking W, Huhn C, Herrmann B, Merkoulov A, Mannhardt J, Jungclas H, Nann T (2009) *Anal Bioanal Chem* 395:1681–1691
132. Ramos A, Morgan H, Green NG, Castellanos A (1998) *J Phys D Appl Phys* 31:2338–2353
133. Morgan H, Hughes MP, Green NG (1999) *Biophys J* 77:516–525
134. Yih TC, Talpasanu I (2008) *Micro and nano manipulations for biomedical applications*. Artech House, Norwood
135. Hughes MP (2000) *Nanotechnology* 11:124–132
136. Kadaksham ATJ, Singh P, Aubry N (2004) *Electrophoresis* 25:3625–3632
137. Lutz T, Donovan KJ (2005) *Carbon* 43:2508–2513
138. Peng H, Alvares NT, Kittrell C, Hauge RH, Schmidt HKJ (2006) *Am Chem Soc* 128:8396–8397
139. Ho SS, Critchley K, Lilly GD, Shim B, Kotov NA (2009) *J Mater Chem* 19:1390–1394
140. Kim WJ, Usrey ML, Strano MS (2007) *Chem Mater* 19:1571–1576
141. Liu FK (2009) *J Chromatogr A* 1216:2554–2559
142. Jellema LC, Mey T, Koster S, Verpoote E (2009) *Lab Chip* 9:1914–1925
143. Jellema L-JC, Markesteijn AP, Westerweel J, Verpoote E (2010) *Anal Chem* 82:4027–4035
144. Blom MT, Chmela E, Oosterbroek RE, Tijssen R, van der Berg A (2003) *Anal Chem* 75:6761–6768
145. Bhagat AAS, Kuntaegowdanahalli SS, Papautsky I (2008) *Lab Chip* 8:1906–1914
146. Kuntaegowdanahalli SS, Bhagat AAS, Kumar G, Papautsky I (2009) *Lab Chip* 9:2973–2980
147. Park JS, Jung HI (2009) *Anal Chem* 81:8280–8288
148. Nam KH, Eddington DT (2010) *J Microelectromech Syst* 19:375–383
149. Yang SK, Cai WP, Zhang HW, Xu XX, Zeng HB (2009) *J Phys Chem* 113:19091–19095
150. Falabella JB, Cho TJ, Ripple DC, Hackley VA, Tarlov MJ (2010) *Langmuir* 26:12740–12747
151. Arnold MS, Green AA, Hulvat JF, Stupp SI, Hersam MC (2006) *Nat Nanotechnol* 1:60–65
152. Planken KL, Kuipers BWM, Philipse AP (2008) *Anal Chem* 80:8871–8879
153. Bai L, Ma XJ, Liu JF, Sun XM, Zhao DY, Evans DG (2010) *J Am Chem Soc* 132:2333–2337
154. Xu T, Jin MS, Xie ZX, Jiang ZY, Kuang Q, Wu HF, Huang RB, Zheng LS (2008) *Langmuir* 24:2281–2283
155. Zhao WY, Lin L, Hsing I-M (2010) *Langmuir* 26:7405–7409
156. Saunders SR, Roberts CB (2009) *Nanotechnology*. doi:10.1088/0957-4484/20/47/475605
157. Enomoto Y, Monjushiro H, Watarai H (2009) *Anal Sci* 25:605–610
158. Hu JH, Ong LB, Yeo CH, Liu YY (2007) *Sens Actuators A* 138:187–193
159. Oberti S, Moller D, Neild A, Dual J, Beyeler F, Nelson BJ, Gutmann S (2010) *Ultrasonics* 50:247–257
160. Xu Y (2008) *J Chromatogr A* 1191:40–56
161. Sperling RA, Liedl T, Dühr S, Kudera S, Zanella M, Lin CJ, Chang WH, Braun D, Parak WJ (2007) *J Phys Chem* 111:11552–11559
162. Gascoyne PRC (2009) *Anal Chem* 81:8878–8885
163. Bachilo SM, Strano MS, Kittrell C, Hauge RH, Smalley RE, Weisman RB (2002) *Science* 298:2361–2366
164. Song LG, Liu TB, Liang DH, Wu CH, Zaitsev VS, Dresco PA, Chu B (2002) *J Biomed Opt* 7:498–506
165. Bulychiev N, Dimberger K, Arutunov I, Kopold P, Schauer T, Zubov V, Eisenbach CD (2008) *Prog Org Coat* 62:299–306
166. d'orlyé F, Varenne A, Gareil P (2008) *J Chromatogr A* 1204:226–232

167. Gallego-Urrea JA, Tuoriniemi J, Pallander T, Hasselov M (2009) *Environ Chem* 7:67–81
168. Lattuada M, Olivo C, Gauer C, Storti Morbidelli M (2010) *Langmuir* 26:7062–7071
169. Thuenemann A, Knappe P, Bienert R, Weidner S (2009) *Anal Methods* 1:177–182
170. Ma PL, Buschmann MD, Winnik FM (2010) *Biomacromol* 11:549–554
171. Bolea E, Laborda F, Castillo JR (2010) *Anal Chim Acta* 661:206–214
172. Celine CL, Lespes G, Dubascoux S, Aupiais J, Pointurier F, Potin-Gautier M (2009) *J Chromatogr A* 1216:9113–9119
173. Schmidt B, Petersen JH, Koch CB, Plackett D, Johansen NR, Katiyar V, Larsen EH (2009) *Food Addit Contam* 26:1619–1627
174. Fernandez B, Costa JM, Pereiro R, Sanz-Medel A (2010) *Anal Bioanal Chem* 396:15–29
175. Isaacson CW, Usenco CY, Tanguay RL, Field JA (2007) *Anal Chem* 79:9091–9097
176. Yamaguchi T, Azuma Y, Okuyama K (2006) *Part Part Syst Charact* 23:188–192
177. Yamaguchi T, Okuyama K (2007) *Part Part Syst Charact* 24:424–430
178. Brewer AK, Striegel AM (2009) *Anal Bioanal Chem* 393:295–302
179. Tiede K, Boxall ABA, Tiede D, Tear SP, David H, Lewis J (2009) *J Anal At Spectrom* 24:964–972