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Field-Flow Fractionation with Inductively Coupled Plasma Mass Spectrometry: Past, Present, and Future

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Abstract

A hyphenated technique of field-flow fractionation (FFF) and inductively coupled plasma mass spectrometry (ICP-MS) is reviewed. The FFF-ICP-MS provides unique information on elemental composition across size or molecular weight distributions. In the early stage of the FFF-ICP-MS development, the technique was found useful for environmental applications. With the growing area of nanotechnology, FFF-ICP-MS has gained increasing interest. A historical background of FFF-ICP-MS is summarized. The key points reported in other previous reviews are summarized. Applications of the FFF-ICP-MS are reviewed to illustrate the potentials of the technique to environmental colloids and nanoparticles. Finally, future prospects of FFF-ICP-MS are considered.

Introduction

Speaking of the analytical techniques, which provide particle size information, field-flow fractionation (FFF) has been one of major players. Field-flow fractionation is suitable for size fractionation of nanometer and sub-micrometer particles or

molecular weight separation of macromolecules of various types, including environmental particulate materials, suspended clays, aquatic colloids, sediments, biological cells, viruses, polysaccharides, and others. When the information of size-based elemental speciation is required, coupling of FFF with atomic spectrometric techniques, such as ICP-OES and ICP-MS is recommended to offer on-line capability with high selectivity. Combination of FFF with a sensitive multi-element detector like ICP-MS allows the determination of the elemental composition across size or molecular weight distributions [1]. This information about elemental size distribution is useful for studying processes involving the formation of colloidal-size particulates and their chemical interactions with associated materials. A specific example is the study of trace element contaminant transport in waterways [2]. Preferential binding of specific elements to proteins can be investigated [3].

Some differences between FFF and chromatography are based on the types of force used to induce analyte retention. In chromatography, forces are highly selective but at the same time are so powerful that they cause irreversible adsorption and structural disruption, including denaturation. In contrast, the FFF retarding forces are much gentler in nature. For that reason, molecular integrity of macromolecules can be retained during separation. Owing to the absence of solid support in FFF, biological activity loss is minimized compared to chromatography where interaction between analyte molecules and stationary phase is likely to occur. Therefore, FFF is considered a gentle separation technique and can be invaluable in separation of macromolecules especially those with weakly bound or labile species. A schematic diagram representing the FFF separation principle is illustrated in Figure 1.

After a decade of FFF-ICP-MS development in 1991, a summary of FFF hyphenated with ICP was documented in a book chapter published in 2002 [4], which

described many features and principles of FFF and highlighted a few examples of FFF-ICP-MS. By that time, only approximately 19 articles related to the use of FFF and ICP were reported. Currently, it has been more than two decades since the first work on FFF-ICP-MS was published. Using the Scopus database from 1991 to present (May 31, 2014), with the keywords "field-flow fractionation" and "inductively coupled plasma" searching for the article or review document type, a total of 119 outputs was found. The publication trend is illustrated in Figure 2. After the first development of FFF-ICP, the technique grew quite slowly in the first decade, and a big jump of approximately 400% increase was observed in the last ten years, suggesting the rapid growth of the technique in the past decade. This might be due to the blooming area of nanotechnology, where the particle size does matter. This expands the horizon of the FFF applications from environmental colloids to nanoparticles (NPs). For the total of 119 articles published, 12 articles are considered as a review type article, contributing to approximately 10% of the total numbers. Therefore, the key points in the previous review articles are summarized in this review.

Furthermore, this review describes about the birth and the status of FFF-ICP-MS in its infancy stage, the use of FFF and ICP in the first decades after the first report of the technique, selected applications of the technique in the second decade for inorganic nanoparticles, and environmental colloids. The future trends of the technique will be forecasted.

The birth and the early stage of FFF-ICP-MS

In 1991, Beckett introduced the first concepts and described initial experience in linking FFF separation techniques with ICP-MS [1]. According to Beckett, FFF yielded accurate and high resolution particle size information for environmental samples (e.g., suspended colloidal matter from rivers and lakes). Owing to the high degree of sample dilution taking place during separation in the FFF channel, a very sensitive analytical technique is needed for subsequent characterization step. In order to gain morphological, compositional, and adsorption capacity information about various natural samples, various detection systems, (e.g., optical microscopy, scanning and transmission electron microscopy, fluorescence, X-ray diffraction, atomic emission spectrometry, and radioactive tracer techniques), have been used [1]. Until one day, Taylor of the U.S. Geological Survey in Denver suggested that ICP-MS might be sensitive enough to give elemental information of the fractionated particles. In addition, ICP-MS could provide multi-element concentration data across the size distribution of the fractionated sample. With these thoughts, the experiment on FFF-ICP-MS was then carried out by Beckett, Taylor, and their coworkers [5, 6]. In 1991, the first off-line FFF-ICP-MS experiments were conducted, in which the FFF separation of suspended matter was carried out in Melbourne (Australia), and the fractionated components were sent to Denver (USA) for off-line elemental characterization by using ICP-MS. In the same year, the first on-line experiments were performed in Denver with the results reported by Taylor et al. in 1992 [5]. In their work, SdFFF was used to separate three model inorganic solid compounds (i.e., lead chromate, aluminum oxide, and iron oxide) suspended in 0.1% tetrasodium pyrophosphate. Another set of samples was surface-water suspended matter collected from the Yarra and Darling Rivers in Australia. Major, minor, and trace element composition of the size-separated colloidal (<1-μm diameter) particulates were

measured in the fractionated samples. In addition, Cd adsorption characteristic in the Yarra River colloid concentrate was studied. Results showed that the amount of Cd adsorbed per mass of solid adsorbate increased as particle size decreased. The authors demonstrated that FFF-ICP-MS was an important tool for studying processes involving the fractionation of colloidal-size particulates and their chemical interaction with associated materials. To avoid the risk of sample clogging, the Babington-type pneumatic nebulizer was used to introduce and nebulize suspended particulates into the ICP torch.

FFF-ICP-MS worldwide

Since its introduction in the early 1990s, FFF-ICP-MS has gradually gained attention. Only approximately 20 publications appeared in the peer-reviewed journals during the first 10 years after the birth of the technique. The technique grew relatively slowly as a consequence of the limited number of equipment manufacturers and their marketing. Only a few FFF manufacturers existed with many distributors worldwide. These manufacturers include: (1) Postnova Analytics, Munich, Germany and Postnova Analytics, Salt Lake City, Utah [http://www.postnova.com]; (2) Consenxus, Ober-Hilbersheim, Germany [http://www.consenxus.de]; and (3) Wyatt Technology Corporation, Santa Barbara, California [http://www.wyatt.com]. Another important reason of why FFF-ICP-MS was of limited use, could be due to the fact that most ICP-MS users were not trained to be FFF practitioners. Likewise, most FFF users were not familiar with ICP-MS. Nonetheless, the situation changed when the nanotechnology began to flourish. Since the FFF-ICP-MS provides great merits and capabilities to solve important problems, more and more research works on FFF-ICP-

MS have been observed. Collaboration between research groups of different expertise can be helpful.

Publications in the area of FFF with ICP are mostly contributed from the researchers in the USA. Spain, Germany, Australia, ranked number two as a major contributor in publishing the applications of this hyphenated technique, as shown in Figure 3. Considering the contribution from various research groups, Colorado School of Mines ranked number one, followed by Monash University as number two, and University of Gothenburg together with Mahidol University as number three (Figure 4). Both oral and poster presentations related to the use of FFF and ICP have been made at many major conferences in the atomic spectroscopic community, including the 2013 European Winter Conference on Plasma Spectrochemistry in Krakow, Poland, and the 2014 Winter Conference on Plasma Spectrochemistry in Amelia Island, Florida, USA.

To summarize the key points related to FFF with ICP-MS detection in the other review articles, the selected contents in those review articles are highlighted in the following section. Then, some selected recent applications of the technique for environmental colloids and engineered nanoparticles are discussed.

Review articles related to FFF-ICP-MS

Various review articles related to the use of FFF and ICP for several investigations were reported. However, the tutorial review, which is directly devoted to FFF and ICP-MS coupling, was reported in 2010 by Dubascoux et al. [7]. This review emphasizes on some key elements regarding the interface between FFF and ICP-MS including the history and practical aspects, and also described about the

applications of the technique for qualitative and quantitative analyses. The interface between FFF and ICP-MS can be a simple connection through a PEEK (polyether ether ketone) or PFA (perfluoroalkoxy alkane) tube between the outlet of FFF system and the sample nebulizer of the ICP-MS system when the flow rate outlets are compatible with the ICP-MS nebulizer. If not, a split connection might be used when the flow rate outlets were too high. The important thing to consider is the issue of particle size selectivity in the nebulizer-spray chamber system. Regarding the applications of the technique, the authors stated that more than 90% of papers were contributed to environmental applications, but only a few papers enlarged the application field with industrial applications, food sciences, and life sciences. The authors summarized some selected applications of FFF-ICP-MS in various applications including environmental, bioapplications, nanoparticles, and others. The feature of flow FFF to allow sample preconcentration was also mentioned to cope with high dilution associated with the inherent principle of the fractionation during FFF analysis.

The other review articles related to FFF with ICP-MS are not directly devoted to this hyphenated technique. However, FFF with ICP-MS was listed together with other techniques for certain applications. The reviews were focused either on engineered nanoparticles or environmental colloids.

For the engineered nanoparticles, various review articles were published [8-10]. The review by Tiede et al. [8] discussed about different analytical techniques available for the detection as well as physical and chemical characterization of engineered nanoparticles in product formulations, environmental matrices and food materials. The authors emphasized the importance of the physical and chemical properties of the engineered nanoparticles within the sample and the chemical

characteristics of any capping/ functional layer on the particle surface. The analytical techniques should be sensitive enough to measure low concentrations, and also minimize sample disturbance. This review categorized a range of analytical techniques into various approaches including microscopy, chromatography, centrifugation and filtration, and spectroscopic techniques, and related techniques. Field-flow fractionation was listed as one of chromatographic related techniques which can provide information on particle number concentration, shape, and size. The FFF technique was discussed as a highly promising technique for size separation of ENPs in complex natural samples. Despite the fact that it is a mild and versatile fractionation technique, careful optimization of carrier composition demands experience and attention should be paid to minimize the membrane interactions. Coupling FFF with ICP-MS is not only possible to quantify different nanoparticles in food, water, biota and soil, but also to characterize or elementally analyze them.

Jiménez et al. [9] described the use of several separation techniques with ICP-MS detection to the analysis of natural and engineered nanoparticles in the environment. They categorized the review into different parts as follows: natural nanoparticles analysis by polyacrylamide gel electrophoresis laser ablation ICP-mass spectrometry (PAGE-LA-ICP-MS); engineered nanoparticle analysis by separation techniques coupled to ICP-MS; and identification and characterization of engineered nanoparticle by single particle detection. To hyphenate with ICP-MS detection, FFF was described as one of separation techniques used for separation, apart from size exclusion chromatography, and hydrodynamic chromatography.

A critical review on the application of plasma spectrometry for the analysis of engineered nanoparticles was given by Krystek et al. [10]. This review covers the use of ICP technique for the determination of ENPs and when applied in various

consumer's products including possible release during use. The authors stated the importance of sample handling and sample preparation for analysis with plasma spectrometry. Care must be taken to preserve size stability. Among all the hyphenated techniques for the analysis of ENPs, FFF coupled with ICP-OES or ICP-MS was also described in the review as a size fractionation technique for the cases of complex mixtures of NPs. With FFF, questions related to membrane-nanoparticle interaction, behaviors of different nanoparticle types in the FFF channel, and the influence of dilution of suspensions during separation should be considered. Also, development of the suitable calibration strategies or the improvement of recovery should be carried out.

Fedotov et al. [11] reviewed the techniques for fractionation and characterization of nanoparticles and microparticles in liquid media. Among all other techniques, FFF was listed as one versatile technique for such purpose. Three modes of FFF separation were mentioned including normal, steric, and hyperlayer. Various sub-techniques of FFF were described. The authors mentioned that the homogeneous samples should be used to obtain representative data, as in FFF the sample weight is less than 1 mg. Additionally, with a very small sample weight, the detection techniques for the analysis of the separated fractions should be highly sensitive. Applications of asymmetrical flow FFF (AF4) coupled online with ICP-MS for study metal associations to different environmental microparticles, nanocolloids, and macromolecules were mentioned.

The applications of flow FFF (FlFFF) for the characterization of natural colloids and natural and manufactured nanoparticles with the emphasis on the detection systems were reviewed by Baalousha et al. [12]. Various detection techniques were mentioned including UV-Vis, organic carbon detector, fluorescence,

ICP-MS and ICP-OES, laser-induced breakdown detection, multi angle light scattering, dynamic light scattering, transmission electron microscopy, and atomic force microscopy. With FlFFF, calculation of the hydrodynamic diameter is based on applying Stokes' relationship assuming that particles are hard spheres. However, not all the cases that the particles are spherical in shape, suggesting the need for a detector giving an independent and complimentary measurement of the particle size distribution. The authors discussed about the use of multi detection approach to help verifying any abnormalities and deviation from theoretical principles of the fractionation, and in some cases to help providing a wide range of information on other particle properties such as composition, concentration, optical properties, interaction with trace metals, and structure. In their review, the operational conditions of FlFFF and the study objective for the analysis of natural colloids and natural nanoparticles were summarized. The natural colloids include extracted humic substances, river and lake waters, estuaries and marine water, wastewater, soil, sediment, and groundwater, atmospheric particles. For the manufactured nanoparticles, measurements of the thickness of surface coating, structure and aggregation behavior were discussed.

The review by Lespes and Gigault [13] also discussed about the use of different hyphenated techniques to obtain multidimensional information for characterization of submicron particles. Hyphenation of an on-line fractionation to one or several complementary detectors was illustrated to be useful. Field-flow fractionation was listed as one of the techniques, which could provide separation before the detection. In comparison with other techniques, the FFF offered advantages in the sense that fractionation power and dynamic range could be easily tuned by varying the field strength. Various sub-techniques of FFF were mentioned.

Detection was classified into the category that could provide particle concentration, particle size, elemental concentration, molecular structure information, and other more specific information. When the information on elemental concentration is sought, inductively coupled plasma spectroscopy was listed as a technique of choice. Various techniques were discussed and compared for their advantages and limitations.

For environmental samples, Contado et al. [14] discussed about different methodologies to fractionate and characterize riverine suspended particulate matter. The review also described about various experimental approaches to perform sampling and preconcentration. These included membrane filtration, tangential flow filtration, centrifugation, and continuous flow centrifugation. For size fractionation, sequential continuous flow centrifugation, tangential flow filtration, split-flow thin-channel fractionation (SPLITT), and FFF were mentioned. Among these techniques, FFF and SPLITT preserved the complexity of the colloidal feature and useful for quantitative approaches to study trace metal distribution on suspended particulate matter, particularly when element specific detectors as GFAAS or ICP-MS were used.

Another review described the various components of FlFFF instrumentation, the nature of the separation process and the theory for translating retention time to relative molecular mass [15]. This review focused on the symmetrical FlFFF subtechnique. The use of frit inlet and outlet feature was described. The frit inlet offers rapid hydrodynamic relaxation of the sample components to reach their equilibrium positions without having to stop the channel flow, thereby avoiding disruption in the channel. The frit outlet was used for skimming off the carrier liquid through the frit materials and thereby allowing the concentrated particles to flow to the detection system, and therefore the detection sensitivity was increased. Regarding the carrier liquid, not only that the ionic strength and the effective dispersion of the

particles need to be considered, it should also be properly chosen to minimize swelling of the membrane, which could lead to non-uniform flows in the channel. Also, care must be taken to avoid potential interference with detector response, interactions with channel materials. Detectors should be selected according to information seek for the sample being analyzed. Two operating modes, including normal and steric/hyperlayer modes were discussed. The applications of FlFFF to environmental and biological matrices and the detection of polymers and inorganic colloids were summarized by listing the analytes, the membrane, the carrier, and the detector used. The authors commented that in order to establish a broader user base with appropriate practical support, the publication of "standard" analytical methods for particular applications might be needed.

The review by von der Kammer et al. [16] presented the current status of FFF as an analytical separation technique for the study of NPs in complex food and environmental samples. The ability to identify the ENPs among the background of all other NPs in the sample is quite challenging. With this, the development of a proper sample-preparation procedure or the use of element specific detector may be considered. Sample preparation should be carried out in such a way that a stable suspension of particles is obtained. The authors commented on different approaches to separate NPs from a certain matrix, such as particle settling, centrifugation, or filtration. Using settling and centrifugation minimized the risks of sample alterations, whereas the membrane filter cannot guarantee that particles smaller than the membrane cut-off indeed pass the membrane quantitatively. For natural NPs which are generally complex, heterogeneous, and present as broad size range, careful optimization of the FFF operating conditions must be carried out in order to obtain the full range of sizing. In addition, optimization must be carried out to separate the

analytical part of the fractogram completely from the void peak, and the mixed modes of elution (normal and steric mode) should be avoided. In general, the carrier liquid should prevent particle dissolution and aggregation. Normally, the carrier liquid should provide sufficient electrostatic repulsion to prevent attachment and loss of the particles to the membrane. The membrane should be chosen to provide enough repulsion between the particles and the membrane surface, but not too high to cause a repulsion-cushion effect causing the early elution of the particles. For the detection, FFF may be coupled with many types of detectors. When the ICP-MS is used, the particle size dependent nebulization and ionization efficiencies in the ICP should be concerned.

Selected applications of FFF-ICP-MS

Considering the applications of FFF-ICP-MS, most of the publications are related to environmental colloids and inorganic nanoparticles. Therefore, some recent applications of the technique are reviewed in the following sections.

Environmental colloids

With FFF-ICP-MS, size-based elemental speciation can be performed. The information on elemental concentration across size distribution information is obtained. FFF-ICP-MS also allows the detection of specific binding of elements to various nanocolloids sub-fractions. This information can be important and useful to gain insight into metal transport and fate. Brief findings of FFF-ICP-MS experiments on environmental applications are summarized as follows:

Bolea and Castillo [17] established the methodology based on asymmetrical flow field-flow fractionation (AF4) coupled with ICP-MS to characterize metal association to environmental particles of a wide range sizes from 1 kDa to 50 μm. In AF4, both steric (microparticle) and normal (nanocolloids and macromolecules) modes of AF4 were performed with prefractionation steps, sedimentation, and centrifugation. Four crossflow programs were used to classify environmental particles into four groups as microparticles eluted in the steric mode (> 1 μm), nanocolloids eluted in the normal mode (15 nm to 1 μm), macromolecules up to 1,000 kDa, and macromolecules less than 100 kDa. To correct for ICP-MS instrumental drift, Cs internal standard solution was mixed with the outlet stream of AF4. Al and Si were mainly found as the microparticles, with which Zn and Pb were significantly associated, as compared to Cu, which was mainly associated with macromolecules.

Worms et al. [18] applied AF4-ICP-MS to examine the association of metals to the colloidal organic matter from wastewater treatment plant. The colloidal organic matter was classified into low molecular mass and high molecular mass fractions. Most metals, including Ag, Cd, Cu, Cr, Mn and Zn, were found associated only with the low molecular mass fraction, suggesting that the low molar mass fraction of the colloidal pool played a key role in controlling the distribution of metals in the colloidal phase. The exceptions included Al, Fe and Pb, which were found to be also associated with the high molecular mass fraction.

Krachler et al. [19] applied the coupling of AF4 with ICP-MS to characterize colloidal iron carriers in peat-draining rivers in North Scotland. The size distribution of natural organic matter determined by FFF-UV (λ=220 nm) was merged with Fe distribution by AF4-ICP-MS. The distribution of Fe was bimodal. The smaller particle size indicated iron complexed by natural organic matter. The second peak

was higher representing the more abundance of Fe, which might be iron oxides. However, for river with high sodium concentration, the signal of secondary Fe species became lower, implying that these iron species were more vulnerable to increase in salinity.

Plathe et al. [20] coupled AF4 to high-resolution ICP-MS to study relationships between trace metals and nanoparticles in contaminated sediment. The signal of several elements including Al, Fe, Si, Mg, Ti, Zn, Cu, Mn, Ba, Pb, Sr, Cr, Ce, La, Co, Th, Bi, Pr, and In were monitored. Al and Si represented the peak maximum at particle size larger than 100 nm. Peak maximum of Fe was at the particle size smaller than 100 nm. The peak maximum of Ti was between that of Al and Fe. The fractograms of Zn, Cu, and Pb were similar to that of Fe and Ti, implying that these elements were held on or in nanoparticle minerals containing Fe and Ti as major elements. The Al-rich minerals were much more depleted in the nanometer size fraction and showed less important role in metal binding.

Neubauer et al. [21] investigated the effects of ionic strength and sample loading on the retention and recovery for size characterization of natural nanoparticles by AF4 coupled with ICP-MS. Wide range of polystyrene sulfonate (PSS) molecular weight standards of 1100-145000 g mol^{-1} were used to cover particle size range of natural organic matter and its aggregates. To obtain reliable results, higher ionic strength carrier solution with lower sample loading were recommended. Ammonium carbonate/ammonium carbamate solution was used as a carrier liquid due to its interference-free ICP-MS property. The Rh solution was added as an internal standard for ICP-MS detection. From the UV-vis fractogram, natural organic matter appeared only one peak, which could bind with about 50% of Fe. However, two

peaks were observed in the Fe fractogram, by which the other peak of Fe was defined as iron-rich nanophase.

Claveranne-Lamolère et al. [22] exploited AF4-ICP-MS together with CE-ICP-MS coupling to characterize uranium–colloid interactions. With AF4-ICP-MS, the colloidal population in soil leachates appeared to be polydisperse, which was identified as aggregates probably constituted of inorganic colloidal Ti, Mg, Ca, Fe, Al-rich and carbon-based particles as well as organic matter. Uranium was associated with the whole colloidal population. Then, CE-ICP-MS was employed to examine the effect of pH on the affinity between uranium and colloid surface sites.

Brittain et al. [23] studied the chemical speciation of U and trace metals in depleted uranium (DU) contaminated soils. Soil samples were extracted with sodium pyrophosphate to remove the organic-bound fraction followed by a further chemical extraction to separate humic and fulvic complexes. The majority of elements (Mn, Fe, Cu, Zn) gave monomodal fractograms for the soils studied, with the exception of Pb and U. Bimodal distribution was found for Pb in the sodium pyrophosphate extract of the Kirkcudbright soil, but Pb exhibited monomodal distribution in the fulvic and humic acid fractions. In the control soil, U was primarily associated with the sodium pyrophosphate fraction with only a small amount of U associated with the humic acid fraction, whereas negligible amount of U was found in the fulvic acid fraction. In the Eskmeals soil, U displayed a monomodal peak in the sodium pyrophosphate fraction, and in the isolated humic fraction, whereas a bimodal peak was observed for the fulvic acid fraction.

Henderson et al. [24] exploited FlFFF-ICP-MS to characterize nanoparticles release during anoxia in grassland soils receiving biannual broiler litter amendments in 12 of the last 15 years. Bimodal distribution was observed at a very small size

fraction and at approximately 400 nm particles or aggregates. High concentrations of Al and Si were found in these fractions with minor contributions from Fe and Ti. The peak of Fe corresponded well with peaks of Si and Al, whereas the peak of Ti was shifted toward later elution times. Nonetheless, P exhibited an elution peak slightly earlier than those of Si, Al, and Fe.

Saito et al. [25] employed FlFFF-ICP-MS to examine size distributions and elemental compositions of nano-colloids in the granitic groundwater. Owing to the relatively low concentration of colloids groundwater, three-step enrichment of colloids was carried out by ultrafiltration, the focusing technique with a large sample injection loop, and the slot flow technique. In most cases, the size distributions of Al, Mg, and Lu were corresponded well with that of organic colloids. Similarly, most of other lanthanide and actinide elements were also shown to be associated with organic colloids, but with smaller size fractions. Sr was found to be associated with Ca-dominant fraction, which might be forming solid solution in $CaCO_3$.

The group of Stolpe reported various examples of FFF-ICP-MS to examine metal binding with environmental colloids [26-28]. They applied on-line coupling of FlFFF to detectors including UV-absorbance, fluorescence, and ICP-MS to examine Fe, P, Mn, Cu, Zn, Pb, and U distributions along with 'nanocolloidal size distributions' (0.5– 40 nm, hydrodynamic diameter) of chromophoric and fluorescent organic matter in the Mississippi river, Pearl river, and the northern Gulf of Mexico [26]. The distributions of Cu and U in all rivers were monomodal in the 0.5–4 nm size range, matching the UV254 size spectrum. In Pearl river, Zn also showed similar distribution with those of Cu and U, but with a broader peak extending into the size larger than 5 nm. For Mn and Pb, the distributions were similar to that of Fe which showed an additional peak in the 5–25 nm size range, in comparison with those of Cu

and U. The distribution of P resembled that of Fe in Pearl river, with P distributed in both the 0.5–4 nm and the 5–25 nm peak. In Mississippi river, on the other hand, it resembled those of Cu and U, by which only the 0.5–4 nm peak could be distinguished.

Further, the technique was also employed to examine Cr, Mn, Fe, Co, Ni, Cu, Zn, and Pb distributions along with 'nanocolloidal size distributions' (0.5– 40 nm, hydrodynamic diameter) of humic-type and chromophoric dissolved organic matter (CDOM) in Alaskan rivers [27]. Similar to UV254 absorbance, carbon, and Fe distributions, during the spring flood, Cr, Co, Cu, Ni, Mn, Zn, and Pb showed one single peak centered at 1.5 nm, and were largely found in the 0.5–4 nm size fraction. During the summer, Cr, Co, Cu, and Ni showed the sharp peak at 1.5 nm, but with a narrower 0.5–3 nm width than during spring flood. For the other elements, their signals were too low to be observed.

In another application, Stolpe et al. [28] used flow FFF-ICP-MS to characterize the binding between nanocolloids (0.5 – 40 nm) with Fe, La, Ce, Pr, Nd, Sm, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu in six Alaskan rivers in the Yukon River basin. Seasonal changes and rivers affected on REE-binding of the nanocolloids. Nanocolloids were classified as 'fulvic-rich', 'organic/iron-rich' and 'iron-rich' fractions. During the spring flood, REEs were high in concentration and were largely associated with <8 nm organic/iron-rich nanocolloids. During summer base flow, the concentrations of REEs were low and REEs were mostly associated with small (<0.5 nm) organic and/or carbonate complexes, probably leached from deeper soil layers.

Inorganic nanoparticles

With the emergence of nanotechnology, the use of nanoparticles has increased tremendously. Inorganic nanoparticles can be detected by FFF-ICP-MS. This review highlights some selected applications of FFF-ICP-MS to various types of inorganic nanoparticles, including silver (AgNPs), gold (AuNPs), titanium dioxide (TiO_2), and other inorganic nanoparticles.

Bednar and coworkers [29] compared the sensitivity and selectivity obtained by various instruments (UV, DLS, ICP-MS, ICP-DRC-MS, and ICP-AES) as an on-line detection system for FFF. In their work, several types of inorganic nanoparticles were investigated, including AgNPs, AuNPs, and quantum dots. Among the detection techniques studied therein, ICP-MS and ICP-AES provided specific and simultaneous detection for AgNPs with detection limit of 10 μg L^{-1} and 100 μg L^{-1}, respectively. For UV detection, even the detection limit of 100 μg L^{-1} was achieved, the technique was not specific and could not differentiate between nanoparticles from different elements. For the DLS technique, the detection limit was as high as 1500 μg L^{-1}. Moreover, the impurities in the suspension or a few large particles skewed the DLS measurement to a larger size. Therefore, the advantages of ICP-MS and ICP-AES as an on-line detection system were emphasized and applied to detect multiple components of nanoparticle systems, including core– shell gold–silver nanoparticles, Cd–Se–Zn–S quantum dots, and silver nanoparticles after sulfidation.

The mixtures of 10, 30, and 60 nm AuNPs with 10, 30, and 60 nm AgNPs were separated by FFF with different detection techniques (UV, DLS, ICP-MS, and ICP-AES). ICP-MS provided the lowest detection limit at 10 μg L^{-1} for both AuNPs and AgNPs compared with ICP-AES (200 μg L^{-1} for AuNPs and 100 μg L^{-1} for AgNPs), UV (400 μg L^{-1} for AuNPs and 100 μg L^{-1} for AgNPs), and DLS (4,000 μg L^{-1} for AuNPs and 1,500 μg L^{-1} for AgNPs).

ICP-MS, ICP-DRC-MS, and ICP-AES were coupled with FFF for characterization of cadmium selenide-zinc sulfide core-shell quantum dots (CdSe-ZnS). For Cd and Zn, ICP-MS provided more detection capabilities over ICP-AES. However, sulfur could not be detected by standard mode ICP-MS (polyatomic interferences), whereas ICP-AES could measure sulfur. Moreover, DRC was used to overcome interferences for sulfur and selenium detection.

Silver nanoparticles

Hoque et al. [30] reported the use of AF4 with on-line and off-line ICP-MS for size characterization and quantitative analysis of AgNPs in aqueous matrices. For online AF4-ICP-MS, indium was selected as an internal standard to correct for signal drift during separation and analysis. External calibration was carried out by injecting a series of AgNPs standard into AF4 system with ICP-MS detection. With this online detection procedure, LOQ of 1.4 ng mL^{-1} (0.1 mL injection volume), LOD of 0.8 ng mL^{-1} were obtained. Three calibration approaches for quantification of AgNPs in water samples were evaluated. These include the external calibration with AgNPs standards, standard addition of AgNPs in the samples injected into the AF4 channel, and flow injection approach using dissolved Ag^{+} standard introduced into the eluent flow after the AF4 system via an on-line injector. The first two approaches provided information on both particle size and concentration, whereas only the information on concentration was obtained from the last approach. Application of the three calibration approaches to a wastewater sample displayed good agreement between the external and standard addition calibration approaches. However, the flow injection approach gave different values, which might be due to the presence of various other insoluble forms of silver (e.g., Ag_2S and AgCl) rather than AgNPs.

Ulrich and coworkers [31] stated the importance of sample handling and preparation on nanoparticle analysis. They studied the effect of dilution, pH, ionic strength on nanoparticle analysis. Two types of nanosilvers were used in this study; AgNPs with citrate stabilized (Ag@citrate, electrostatic stabilization) and AgNPs with PVA stabilized (Ag@PVA, steric stabilization). With sample dilution, increasing of particle size was observed for both types of AgNPs, which might be due to an alteration of the ratio between nanoparticle and coating or functionalization agent bound to its surface. The effect of ionic surfactant or electrolyte addition into the carrier liquid was also investigated. Adding sodium nitrate into the carrier liquid resulted in increasing in size of both types of silver nanoparticles. The authors suggested that addition of ions might change the electrostatic force between coating or functionalization agent and nanoparticle core, which in general the sterically stabilized nanoparticles should be less sensitive to ionic strength changes than the electrostatically stabilized ones. Similarly, the effect of pH changes was more impact on the electrostatically stabilized than the sterically stabilized nanoparticles. The time dependent curves showing the change in particle size upon pH change implied the kinetics of the agglomeration behavior. For Ag@PVA a relatively steep increase was observed after 120 minutes, whereas it took only after 60 minutes for Ag@citrate. Moreover, the charge of nanoparticles also affected on separation efficiency and recovery rate in FFF-ICP-MS analysis. Shift in the retention time might be expected with different particle-membrane interaction.

Bolea et al. [32] reported the hyphenated techniques of AF4 and ICP-MS for the characterization and quantification of AgNPs standards and AgNPs in consumer products. Various parameters including the mobile phase composition (pH, ionic strength, and the presence of surfactants), the injection/focusing time, and the

crossflow were optimized. Different compositions of nonionic (Tween 20) and ionic surfactants (SDS) were compared in term of the colloid stabilization based on the steric and electrostatic repulsion. Nonionic surfactant offered the shorter time elution peak with narrower profile, which might be due to weaker repulsion forces among AgNPs, and thereby the particles were focused into a narrow area. Nonetheless, with nonionic surfactant, three different AgNPs standards (10, 20, and 80 nm) eluted at the same retention times, suggesting that nonionic surfactant was not an appropriate carrier liquid under this condition. In contrast, ionic surfactant promoted electrostatic repulsion forces, leading to longer retention times of the AgNPs. The best sample recovery for all AgNPs standards was observed when 0.01% SDS at pH 8 was used as a carrier liquid. Increasing ionic strength of the carrier liquid led to the reduction in sample recovery, as the repulsion between AgNPs and the membrane surface was shielded, leading to nanoparticle adsorption on the membrane surface. With ultrapure water as a carrier liquid, conditioning of the membrane surface was necessary by repeated injections of concentrated AgNPs to reduce adsorption, by which recoveries of 98±5% were obtained when four injections of AgNPs were applied to condition the membrane. Between the two commonly used membranes, poly(ethersulfone) or PES membrane provided higher recovery for AgNPs, as opposed to regenerated cellulose membrane. With optimum condition, FFF channel calibration was performed by using various AgNPs standards with the particle size between 10 to 80 nm, and consequently the method was applied to characterize particle size of AgNPs in antiseptic and dietary supplement. For element detection and quantitation purpose, ICP-MS was used. The results obtained from direct nebulization were agreeable with those from the digested sample analysis.

Poda et al. [33] reported the use of FFF-ICP-MS for characterization of AgNPs mixture and applied to biological media samples. In their work, a symmetrical FlFFF equipped with 10 kDa regenerated cellulose membrane was used with 0.025% FL-70 in the presence of 0.025% sodium azide as carrier liquid. NIST-traceable polystyrene bead size standards (20, 50, and 100 nm) were used for FlFFF channel calibration. The recoveries of 88-89% were obtained for the three sizes of AgNPs at 10, 40 and 70 nm. The recovery of less than 100% might be due to the interaction of particles with FFF membrane and particle loss during ICP-MS sample introduction. The sizes of AgNPs obtained from FFF analysis agreed well with the results from DLS, but larger than the TEM sizes, reflecting that the FFF and DLS techniques measure the hydrodynamic particle rather than the primary particle size. Injection of a mixture containing 10, 40, and 70 nm AgNPs at concentrations of 67 μg L^{-1} showed clearly defined peaks, although baseline resolution was not achieved. With increase in particle size, the noise signals were significantly increased. The authors hypothesized that this might be due to the delivery of larger amounts of Ag per particle unit into the plasma/detector system. At the concentration down to 6.7 μg L^{-1} of mixed AgNPs, three peaks were quite noisy, but still sufficiently defined. However, increasing the cross flow from 1 to 1.1 mL min^{-1} improved the peak separation with minimal reducing sensitivity and minor peak broadening. In addition, the study of biota exposure to AgNPs was carried out. The freshwater oligochaete, *Lumbriculus variegates,* was exposed to PVP-coated AgNPs spiked sediment. The tissues were extracted after 28 days exposure by deionized water using sonication. The authors found that AgNPs became bigger at 46 nm after biological exposure compared to the original size of 31 nm, which might be resulted from coating of the particles with proteins or other biological molecules. Alternatively, the PVP coating

might be removed as a result of biological mechanisms or abiotic reactions in the soil exposure medium, resulting in aggregation of the destabilized silver particles.

Coleman and coworkers [34] used FFF-ICP-MS to examine the effects of particle size and the coating on the bioaccumulation and depuration of AgNPs within the gut cavities of aquatic invertebrates (*L. variegates*). Prior analysis, the *L. variegatus* was homogenated and sonicated for 1 h in deionized water. After 48 h of depuration, FFF-ICP-MS fractograms showed a fraction of AgNPs which were smaller in size than the original particle sizes. This result was agreeable with the data from single particle (SP)-ICP-MS analysis performed for the collected fractions from FFF. The analysis of the collected fractions at the shouldered void peaks from FFF with the SP-ICP-MS revealed that this fraction could be either dissolved Ag, small particles, or Ag associated with organic moieties, such as proteins recovered from the tissue with water sonication extraction. However, all dissolved Ag should be removed by cross flow and filtered off through the membrane before reaching the detector. This study illustrated that with FFF-ICP-MS and SP-ICP-MS, AgNPs could be retained in the *L. veriegatus* for longer than the standard 6 h depuration period, as indicated in the recommended procedure by USEPA for bioaccumulation testing.

Another work by Poda et al. [35] was to examine the effect of UV irradiation on the stability of AgNPs using FFF-ICP-MS. The 50 nm polyvinylpyrrolidone-capped AgNPs in the presence and absence of dissolved organic carbon (DOC) were examined. AgNPs were prepared in three different media, including deionized (DI) water, moderately hard reconstituted water (MHW), and MHW with DOC. The ICP-MS response of AgNPs in MHW was decreased when compared with AgNPs in DI water, as MHW triggered particle aggregation by charge neutralization. However, adding 4 mg L^{-1} DOC solution in the MHW caused the increase in the ICP-MS signal,

as the DOC solution consisted of negatively charged carboxylates that increased nanoparticle dissolution by scavenging and complexing Ag^+ and possibly reducing Ag^+ to Ag^0. To mimic 24 h exposure of AgNPs to sunlight, photolysis with sixteen 14 W black lamps was set up for approximately 1 h. The photolysis resulted in a decrease in average particle size of AgNPs, which might be due to the photo-induced oxidation of the PVP and dissolution of metallic silver. Moreover, it was observed that particle size of AgNPs in MHW and DOC/MHW were also decreased after photolysis reaction. Not only particle size was decreased after adding DOC component in AgNPs solution, the overall particle concentration was also decreased, which might be caused by the oxidation of silver to Ag_2O followed by dissolution. In addition, the toxicity of AgNPs under light exposure was investigated using the standard zooplankton model.

Loeschner et al. [36] developed a method based on the use of AF4-ICP-MS for the analysis of AgNPs in chicken meat. To mimic a possible scenario where AgNPs may migrate from an antibacterial food packaging material into meat, AgNPs stabilized with polyvinylpyrrolidone (PVP) were spiked into chicken meat. Then, enzymolysis by Proteinase K for 40 min at 37 C of the homogenized AgNPs spiked chicken meat sample was performed to degrade protein to peptides and amino acids. The fractogram of the enzymatically digested meat sample containing AgNPs displayed one large peak with two smaller peaks eluting close to the void volume. The large peak was assigned to AgNPs which eluted earlier than the pristine AgNPs without meat. This earlier elution hampered the accurate determination of the particle size distribution based on retention times.

Bolea et al. [37] applied AF4-ICP-MS for investigation of changes in AgNPs particle size incubated in culture medium and cells to gain an insight into nanotoxicity.

Particle size of AgNPs was bigger than the original size upon incubation in the culture medium, suggesting the occurrence of protein corona formation around the AgNPs. Using the operating condition to accommodate protein fractionation (19, 53, 113 kDa), Ag signals appeared at the same retention time as the proteins suggesting that AgNPs were partially oxidized to Ag^+ which could associate with proteins present in the culture medium. The association of AgNPs with HepG2 cells was examined by using AF4-ICP-MS after alkaline solubilization. The fractograms revealed the presence of AgNPs, but with a smaller size than the original size, implying that a dissolution process might occur. However, the presence of AgNPs peak provided the evidence of AgNPs association with the cells.

Gold nanoparticles

Schmidt et al. [38] reported the use of FFF coupled with ICP-MS for separation and quantification of 10, 20, and 60 nm AuNPs mixtures. The mean recoveries of 50%, 95%, and 67% were observed for 10, 20, and 60 nm AuNPs, respectively. The incomplete recoveries were due to possible adhesion of AuNPs on the membrane surface during separation process. The limits of detection were varied between 0.02 ng Au to 0.4 ng Au depending on nanoparticle diameter. The LODs were increased with increasing particle size. AuNPs of 10 nm and/or 60 nm were injected to sixteen female Wistar rats. After 24 h, the livers were taken out and solubilized in tetramethylammonium hydroxide. With ICP-MS detection using Rh as an internal standard after microwave assisted wet ashing, the recoveries of AuNPs from rat livers were ranging from 86% to 123% of their total Au content. However, the 10 nm or 60 nm AuNPs could not be observed by FFF owing to the association of AuNPs with remains of liver tissue.

Gray et al. [39] compared the performances of hydrodynamic chromatography (HDC) and AF4 coupled with ICP-MS for the analysis of AuNPs. The resolution, recovery, and detection limit obtained by the two techniques were evaluated. Two types of AuNPs, which were tannic stabilized- and citrate stabilized-AuNPs of 5, 20, 50, and 100 nm, were examined. The resolution obtained from HDC was poorer than that from AF4, despite the fact that better resolution of AF4 over HDC came with the drawbacks of longer run time. With both techniques, different surface coating on the AuNPs showed variability in the relationship between time and diameter under identical operating conditions, implying that size calibration should be performed by using the particles with coatings that caused a similar retention behavior to that of the unknown sample. This problem should be paid great attention as in the environmental or biological systems, NPs were likely to accumulate coatings on their surface. Considering the sample recovery during separation process, HDC provided higher recovery than AF4 as particle sorption on the separation membrane was the main cause of sample loss. The limits of detection for both techniques were approximately 5 μg L^{-1}.

Titanium dioxide nanoparticles

Owing to its several interesting properties, i.e., insoluble; high stable; nonreactive; thermally stable; nonflammable; low cost; and environmental friendly, TiO_2 nanoparticles are used in many applications. Various research groups reported the use of FFF with element specific detectors for size characterization of TiO_2 nanoparticles, particularly in cosmetic products.

In 2008, Contado and Pagnoni applied FFF with off-line ICP-AES detection for size characterization of TiO_2 in commercial sunscreen lotion [40]. TiO_2 particles

showed a strong affinity for each other and, in some cases, even for the accumulation wall of the FlFFF channel (membrane). In the case of commercial sample, Ti concentration profiles obtained by ICP-AES were agreeable with those from the UV detector. The authors mentioned that different sunscreen formulations required different sample preparation methods. In 2010, the same researchers proposed the use of square wave voltammetry as a lower cost technique to quantify the Ti concentration in commercial foundation creams [41]. In their study, the results obtained from the square wave voltammetry were compared with ICP-AES. With square wave voltammetry, the presence of interfering elements, such as Pb^{2+} and Cu^{2+} resulted in the unexpectedly lower values of Ti concentration as compared to the values obtained by ICP-AES. Therefore, ICP-AES was used as an off-line detector for the fractions collected from FFF. Furthermore, SdFFF and FlFFF were used to investigate the size of TiO_2 nanoparticles in the commercial foundation creams after solvent extraction. The mixture of water : methanol : hexane as a ratio of 1 : 4 : 1 was an optimum extractant because the higher amount of methanol better dispersed the foundation, and was evaporated more rapidly than water. With SdFFF analysis, the void peaks for all six concentrated suspension samples were significantly high, which might be contributed from some particles with size smaller than 100 nm or bigger size with density lower than 4 g mL^{-1}. To analyze the smaller particle size fraction, FlFFF was employed and this sub-technique was density independent. With FlFFF, particles of about 2-3 nm and about 15 nm were detected in the samples.

Samontha et al. [42] reported the coupling between SdFFF and ICP-MS for size characterization of TiO_2 nanoparticles in sunscreen samples after hexane extraction to remove organic components from the samples. Sunscreen products of various sun protection factor (SPF) values were used as samples. The particle size

distributions of titanium dioxide in most sunscreen samples investigated in this work were larger than 100 nm, which might be attributed to the secondary particles. The authors also carried out quantitative analysis of titanium in the samples by comparing the results obtained from the on-line SdFFF-ICP-MS and those from the off-line ICP-MS determination of titanium after acid digestion. The concentrations of titanium found from both methods were in good agreement.

Nischwitz and Goenaga-Infante [43] described the first systematic comparison and optimization of extraction methods for titanium dioxide nanoparticles in sunscreen samples. They used FlFFF with on-line ICP-MS for size characterization of titanium dioxide nanoparticles in sunscreens after defatting of the sample with hexane followed by bath sonication with an aqueous extractant. With this extraction procedure, stable suspensions of secondary titanium dioxide particles were obtained. To obtain the primary particle size, further addition of a small amount of hexane to the aqueous extractant was recommended to disaggregate the particles. The developed sample preparation procedure prior to FFF-ICP-MS was applied to analysis of commercial sunscreens with various sun protection factors.

Another recent published work on the use of AF4-ICP-MS for TiO_2 nanoparticles analysis in cosmetic and food products is given by López-Heras et al. [44]. In their work, the experimental parameters affecting NPs separation and elemental quantification by AF4-UV/ICP-MS were optimized and discussed. Polystyrene latex standards of three known sizes (22, 54, and 100 nm) were used as calibrants. However, by collecting the fractions analyzed by AF4 to further characterize by TEM, it was found that erroneous results might be obtained when using the latex standards for calibrating rutile TiO_2 NPs size, due to the high aggregation state of TiO_2. To reduce agglomeration, the use of ultrasound energy was

explored. Then, AF4-ICP-MS was applied to detect TiO_2 NPs in moisturizing cream, sugar glass, and coffee cream. However, Ti signal was negligible in food extracts, suggesting that although TiO_2 was commonly used as anti-caking agent and food additive, the compound might not be present as NPs.

Ulrich and coworkers [31] examined the effects of pH, ionic strength, and dilution on the analysis of polyacrylate functionalized TiO_2 nanoparticles. The size of TiO_2 nanoparticles was not significantly changed after 1:50, 1:100, 1:500, 1:1000, and 1:5000 dilution. The change of TiO_2 particle size was also insignificant by changing ionic strength with sodium nitrate addition. Nonetheless, pH change affected on the particle size of TiO_2 nanoparticles. In this case, hydrolysis or the other chemical interactions could influence on crosslinking or binding between polymer coating component of TiO_2 nanoparticles stabilized by polyacrylate.

Other inorganic nanoparticles

Pace et al. [45] applied FlFFF-ICP-MS for characterization of four CdSe/ZnS quantum dots including 2 nm QDs (green-emitting) and 5 nm QDs (red-emitting) with two different surface coatings, i.e., polyethylene oxide (PEO); and 11-mercaptoundecanoic acid (MUA). The isotopes monitored included ^{64}Zn, ^{82}Se, and ^{114}Cd using ^{45}Sc and ^{209}Bi as internal standards for all the samples. However, instrument drift during FlFFF-ICP-MS analysis was not observed. Therefore, all elemental concentrations were not normalized by internal standard intensities. The signals from ^{64}Zn, ^{82}Se, and ^{114}Cd exhibited similar hydrodynamic diameters with the size of 23, 24, 24, and 29 nm for red MUA, green MUA, red PEO, and green PEO QDs, respectively. However, their elemental compositions were significantly different. The MUA-coated QDs provided a very huge Cd signal compared to Se

signal. The ratios of Cd/Se were 2.1, 23, 1.3, and 11 for red PEO, red MUA, green PEO, and green MUA QDs, respectively. These QDs were subjected to acute toxicity test by exposing to *Daphnia magna* for 48 h.

Huang et al. [46] incubated Be-associated materials in synthetic lung fluid (SLF) to study their dissolution and nanoparticle generation behaviors. To mimic inter- and intra- cellular environment in lung tissue, pH of SLF was adjusted to 4.5 and 7.2, respectively. AF4-ICP-MS was utilized for size characterization of the particles in incubated samples from days 0, 4, 8, 32, 49, and 149. Cs was selected as internal standard for ICP-MS elemental quantification. The time-dependent dissolution and particle generation were investigated for $Be(OH)_2$ suspension (pH = 4.5). No signals of Be, Al, and Si were detected in the size less than 450 nm for $Be(OH)_2$ suspensions on day 4 whereas they were found at 10-60 nm by day 32. However, the signal intensities of 10-60 nm particles were decreased whereas the larger particles were formed suggesting the presence of particle aggregation in SLF. Similar trend was observed at day 149. For all Be-associated materials (bertrandite-containing ore, beryl-containing ore, frit, $Be(OH)_2$), they could significantly produce nanoparticles with the size distribution of 10-100 nm. However, less nanoparticles were observed for BeO, for which ionic Be were significantly produced after SLF incubation.

M-M et al. [47] exploited FlFFF-ICP-MS to study particle size characterization of selenium nanoparticles stabilized by pectin, mixed alginate/pectin, ovalbumin, and β-lactoglobulin. Quantification of selenium was performed and indicated that a total of approximately 88–103% of the original selenium nanoparticles was detected during fractionation. These nanoparticles were subjected to incubation in gastrointestinal conditions, both in enzymatic and non-enzymatic

media. Particle size distributions shifted differently depending on the type of the stabilizing agent used in the preparation of selenium nanoparticles. Despite the shift in particle size distribution, more than 90% of selenium was still present in nanometer range after gastrointestinal digestion.

Heroult et al. [48] reported the use of AF4-ICP-MS together with multi-angle light scattering (MALS) and offline with transmission electron microscopy (TEM) with energy-dispersive X-ray analysis (EDAX) to perform size-based elemental quantification and size estimation of silica (SiO_2) nanoparticles in coffee creamer. For optimization, SiO_2 of 12 nm was used and baseline separation was achieved with the cross flow rate of 2 mL min^{-1}. Increasing the cross flow rate higher than 2 mL min^{-1} resulted in better separation with the expense of poorer recovery. Interestingly, the elution time of 12-nm SiO_2 was shorter than that of the 10-nm AuNPs calibrant. The authors hypothesized that the different elution behaviors between the two particles might be caused by differences in particle–membrane interactions between these particles. For the analysis of SiO_2 in coffee creamer, sample preparation was carried out by mixing the coffee creamer with water at different temperatures (40 °C and 60 °C) to mimic how the coffee was prepared in real life. Silicon fractograms obtained by FFF-ICP-MS for coffee creamer suspensions showed no significant effect of the water temperature on the particle size distribution of silica nanoparticles in coffee creamer extracts.

FFF-ICP-MS: concluding remarks and future challenge

Field-flow fractionation-ICP-MS is regarded as a size-based elemental speciation tool, since it produces information on chemical composition across the size

distribution of particulate and macromolecular samples. Various important applications of FFF-ICP-MS on size characterization of environmental colloids and nanoparticles were reviewed showing that the technique provides unique and new information, which cannot be gained otherwise. The technique shows great potential to revolutionize our understanding of trace metal-colloid interactions in environmental studies. The chemical composition as a function of particle size can be determined and adsorption characteristics can be investigated. Also, it provides information about bio-inorganic metal binding with macromolecules. The FFF-ICP-MS can be used to study the competitive binding of several metal ions to particular macromolecules. Regarding the engineered nanoparticles, applications of FFF-ICP-MS for the detection of inorganic nanoparticles in complex matrices, such as food or environmental samples, are useful. Further, FFF-ICP-MS can be used to gain more understanding of how nanoparticles interact with biological system, to provide the evidence of bioavailability and toxicity of nanoparticles.

Considering the applications of FFF to nanoparticles, the difficulty is arisen from the variability in the retention behaviors of the same nanoparticle core with different coatings. Different particle/membrane interactions can cause shift in elution time of the same size particles, and also variation in sample recovery during separation process. The former leads to incorrect interpretation of particle size information, whereas the latter hinders the quantitative analysis of the nanoparticles. Solving these problems can be a challenge. To enable accurate interpretation of particle size, the use of single particle (SP)-ICP-MS in combination with FFF-ICP-MS is helpful. The works on FFF-ICP-MS with SP-ICP-MS can be found in several publications [34, 46, 49, 50], and many more publications on these techniques are expected. The use of matrix matched standard or the standard particles of the same

coating is suggested. Nonetheless, it is difficult to match the type of particle coating between the standard and the unknown sample in practice. To overcome this problem, we therefore propose the concept of matrix modification by addition of appropriate amount of coating or stabilizing agent in the carrier liquid. The experiments to prove this concept are now being conducted in our laboratory.

The main goals of analytical chemistry are identification and quantification. Most FFF-ICP-MS works have reported only species identification. Although the report on the quantitation by FFF-ICP-MS has been found, the quantitative information has not been emphasized in most of the published articles. Elemental speciation of macromolecular compounds is often involved with large carbon-rich molecules [51, 52]. The presence of high carbon contents can hinder the accuracy of trace element determination by ICP-MS. As previously reported, carbon concentration of higher than 1,500 mg L^{-1} could cause serious non-spectroscopic interferences [53]. Although no clear evidence exists, varying compositions in fractionated peaks influence the ionization efficiency in the plasma and therefore can hinder exact external calibration of fractograms. The prohibitive factor to obtain accurate quantification of elements in colloids or macromolecules is a lack of standards. To obtain accurate quantitative information of trace elements bound to macromolecules or particles, standards or standard reference materials must be produced for a particular sample type. Alternatively a method, which can overcome the matrix effects, must be used. Another clever way to achieve high accuracy is to use ICP-isotope dilution MS (ICP-IDMS), where sources of systematic error are well understood and can be controlled. Quantification by the isotope dilution analysis is based on the measurement of an isotope ratio and not on the absolute ion intensity. Application of an on-line FFF-ICP-IDMS should be considered by adopting the

concept of an on-line HPLC-ICP-IDMS suggested by Heumann et al. [54-59]. Two different spiking modes are possible, one using species-specific and another using species-unspecific spike solutions of isotope-enriched labeled compounds. The species-specific mode is only possible for element species well-defined in their structure and composition, whereas the species-unspecific mode could be applied in all cases where the structure and composition of the species are unknown. In the case of FFF-ICP-MS work, the latter approach, species-unspecific mode, is suggested. With the latter approach, equilibration of isotopes on line is not necessary. Instead of the HPLC column, the FFF channel can be used. This concept has been successfully demonstrated recently by Meermann et al.[60] for quantification of AgNPs. The on-line isotope dilution appears to be an ideal approach for accurate quantification of speciated elements. Nevertheless, the method is limited to only analyte elements with various sufficient isotopes. Monoisotopic elements (e.g., Al, As, Au, and Mn) cannot be quantified by this approach.

Since the FFF-ICP-MS provides great merits and capabilities to solve important but unusual problems, analysts can expect to see more and more research works on this hyphenated technique. Collaborative work between analytical chemists and biochemists, biologists, botanists, and nutritionists is suggested. With the developed FFF-ICP-MS technique, the role of trace metals and their isotopes in nutrition, health and disease can be studied. With the use of enriched stable isotopes, differentiation between externally spiked elements and endogenous pools makes the investigation of the exchange between endogenous and exogenous elements of metal-containing macromolecules possible.

In most of the FFF-ICP-MS publications, only SdFFF and FlFFF sub-techniques have been used. Coupling between ElFFF and ICP-MS may be seen in the

future, as ElFFF has been shown able to separate nanoparticles [61, 62]. Since the separation in FFF is driven by physical force, less chemistry is involved in FFF compared to SEC or other chromatographic separations. To obtain elemental size separation without destroying the structure integrity of macromolecules or colloids, FFF-ICP-MS holds the best promise. Despite other advantages, FFF also suffers from limited separation resolution. Depending upon each FFF subtechnique, resolution might be improved by optimizing parameters governing fractionation power. Analytical chemists can expect to see multidimensional FFF, especially FFF-CE, add to the specificity of their elemental analyses. Multidimensional, hyphenated systems like FFF-CE-ICP-MS or FFF-HPLC-ICP-MS might be necessary to gain detailed information on clinical and environmental studies, where elemental size distribution and its functionality information are sought. This idea was demonstrated by Dubascoux et al. [63] to analyze the fractions collected from AF4 for further speciation analysis of organotin in environmental colloid by using off-line head-space solid-phase microextraction-gas chromatography with pulsed-flame photometric detection. The work on combination of FFF with other speciation techniques to provide more insightful information on elemental speciation should gain more interest. Looking to the future, FFF-ICP-MS can indeed offer a lot more as one might imagine. With our entrance into the "proteomics era", it is essential to develop ideal assay for detection and quantification of proteins [64, 65]. The use of FFF-ICP-MS in combination with FFF and electrospray ionization mass spectrometry may be an additional approach for metalloproteomics.

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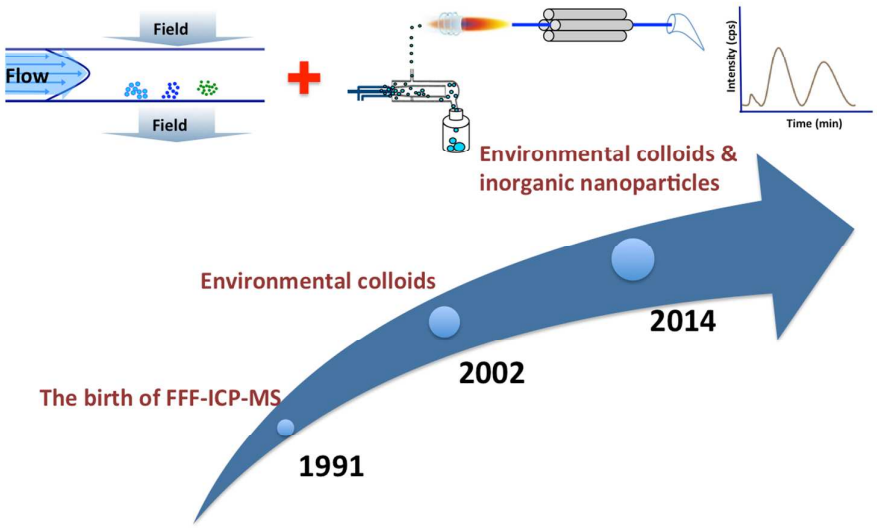
Figure 1 FFF principle and sub-techniques.
In FFF, separations are achieved within a flat open channel with a rectangular cross-section and triangular end pieces where the sample and carrier liquid enter and leave. The essence of FFF is to apply a force perpendicular to the channel flow stream, which is a parabolic flow profile. This force drives macromolecules or particles toward an accumulation wall where they encounter slow-moving laminar fluid streamlines. Fractionation is achieved by the balance between the applied field force pushing the sample particles toward the accumulation wall and the diffusivity of sample particles acting against the field force. Smaller species diffuse away from the accumulation wall into the carrier stream faster than larger species. Thus, various size sample components are driven into different average positions axially and laterally in the channel flow. Consequently, each sample component leaves the channel at a different time related to its diffusion coefficient and thus molecular weight. These primary driving forces can be generated by a number of fields or gradients, resulting in numerous FFF sub-techniques that are generally applicable to different materials.

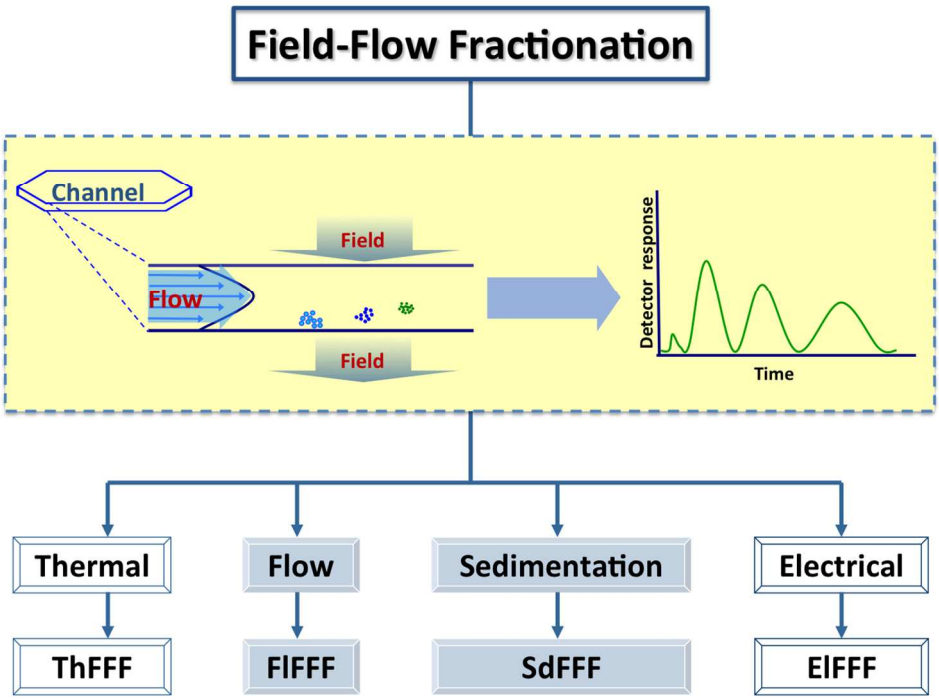
Figure 2 Publication trend on “FFF” with “ICP” since 1992.

Figure 3 Publications on “FFF” with “ICP” since 1992 as classified by country.

Figure 4 Publications on “FFF” with “ICP” since 1992 as classified by the institution.



Historical background, recent applications, and future trends of field-flow fractionation-inductively coupled plasma mass spectrometry



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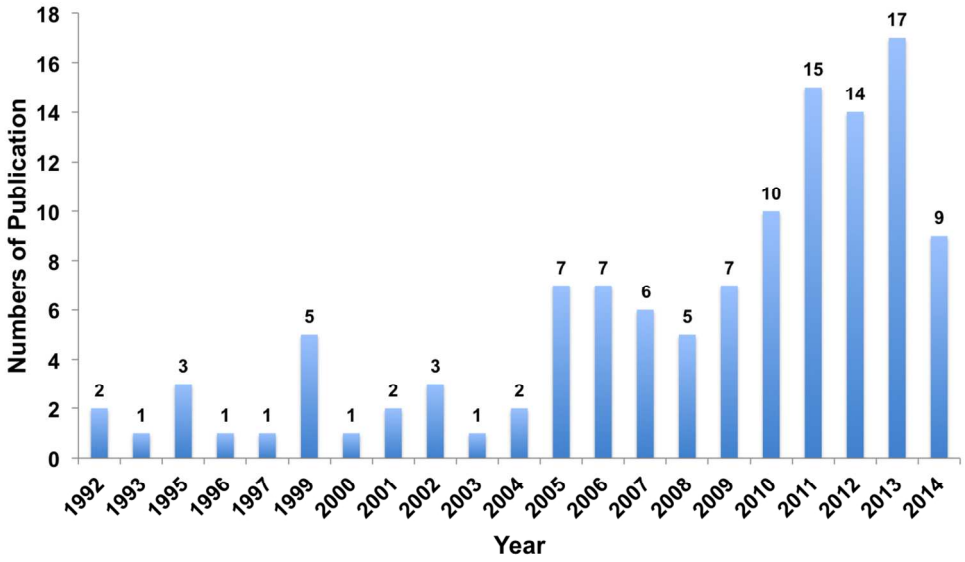

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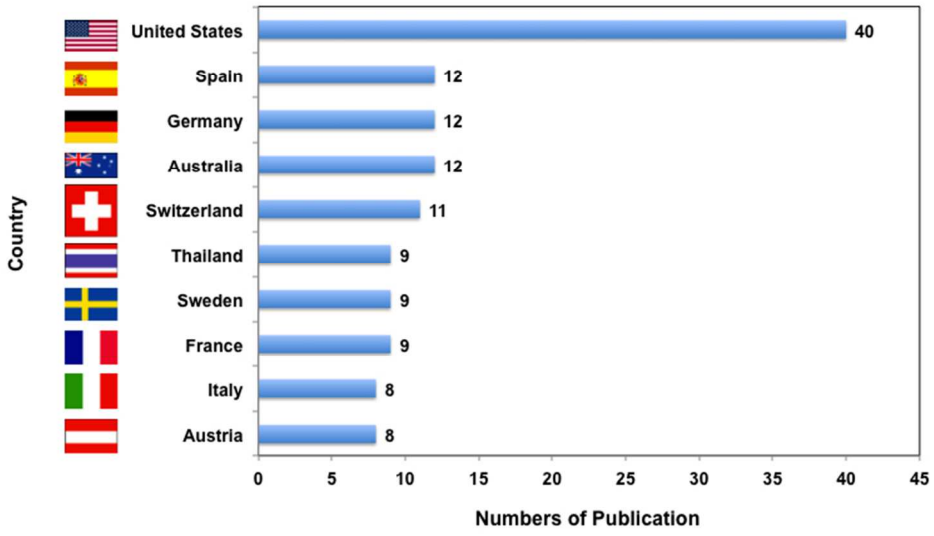

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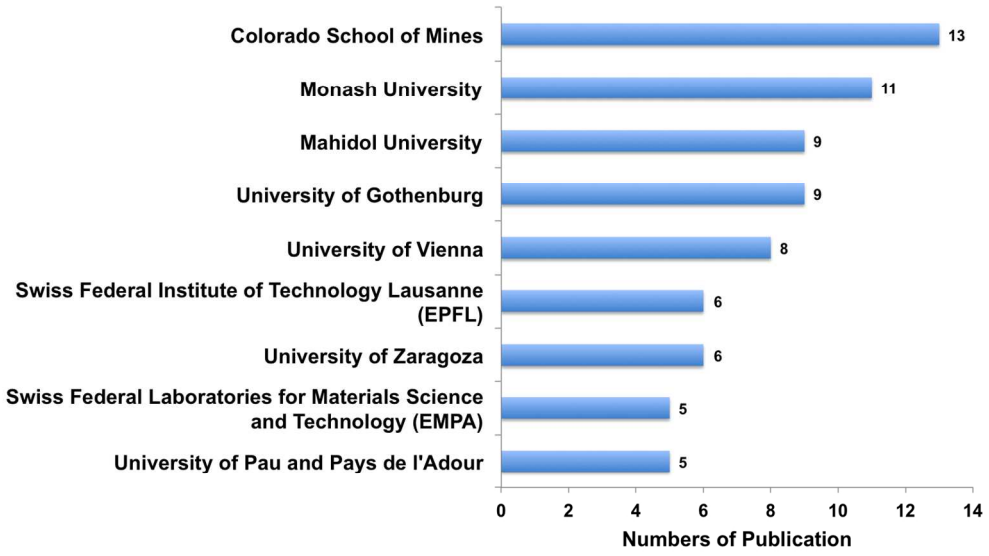

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