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Exploring environmental nanobiogeochemistry using field-flow fractionation and ICP-MS-based tools: background and fundamentals

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The recent application of sophisticated instrumentation and novel experimental techniques to environmental systems has driven the study of natural nanoparticles and nanoparticle systems towards new horizons. Moving beyond the detection of engineered nanoparticles in natural systems, these technologies create new knowledge about the composition, behaviour, and functions of natural nanoparticles as individual entities and particle systems. In this tutorial review, we define the emerging field of environmental nanobiogeochemistry and describe the fundamentals, optimization, advantages, and disadvantages of field-flow fractionation and ICP-MS-based techniques for advancing our understanding of natural nanoscale particles and particle systems. The companion perspective Exploring environmental nanobiogeochemistry using field-flow fractionation and ICP-MS-based tools: progress and frontiers describes the progress and frontiers in this research area using case studies drawn from a range of published and unpublished data spanning diverse environmental systems. Thus, by combining necessary background with the most recent findings and key challenges, these contributions provide key knowledge for new and established researchers entering this exciting field and lay the groundwork for future research.

Environmental significance

The use of field-flow fractionation and ICP-MS-based techniques to study natural nanoparticles is hindered by challenges in their operation and the interpretation of the corresponding data, potential artefacts arising from the nature and complexity of environmental systems, and lack of understanding about the knowledge they can provide. However, recent advancements have opened wide research frontiers that can substantially advance the understanding of natural nanoparticle composition, behaviour, and functions. This tutorial review enables researchers to apply these techniques to natural nanoparticle systems by combining key background information with a review of challenges in optimization, advantages, and disadvantages. Combined with the companion perspective article Exploring environmental nanobiogeochemistry using field-flow fractionation and ICP-MS based tools: progress and frontiers, this review provides the tools and knowledge for new and established researchers to advance the emerging field of environmental nanobiogeochemistry.

1. Background

Natural nanoparticles (NNPs) are major constituents of aquatic systems, with a size of ≤100 nm in at least one dimension, and comprise a portion of the size range of aquatic colloids (≥1 D with a size between 1–1000 nm).[1] These NNPs share important environmental roles with particles in the dissolved (<450 nm, operationally defined by filtration) and small particulate (1–5 μm) fractions. While they may be composed of organic, inorganic, or mixed organic–inorganic materials, most natural NNPs and larger particles have mixed composition, highly diverse surfaces, and the corresponding capacity to adsorb, bind, or complex a wide range of ions, small molecules, and small particles. This high adsorption capacity, coupled with their small size and resistance to settling, contributes greatly to their environmental significance.[2]

The distribution of suspended particles with a size of <1 μm follows the Pareto or "power law" distribution $n = Ad^{-\beta}$, where β is close to 3, meaning that the number (n) of

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suspended particles increases logarithmically as their diameter (d) decreases.[3,4] This distribution is influenced by the processes governing agglomeration and settling, which also contribute to the fractal structure of aggregates.[5,6] Fractal structures composed of macromolecular organic matter such as humic substances or proteins and/or small particle clusters provide larger surface areas compared with particles of the same or even larger sizes that have spherical, quasi-spherical or crystalline shapes, such as primarily inorganic hematite or goethite. Indeed, particles <200 nm contribute more than 55–71% of the total surface area of particles <5 μm.[7] Their large surface area and diverse functional groups facilitate adsorption, making NNPs significant vectors for transporting contaminants and nutrients in surface waters, soils, and groundwater.[1,8,9] Their dynamic interactions with adsorbed organic matter, contaminants, and nutrients also influence bioaccessibility and bioavailability.[10–14]

Despite their importance for governing the transport and bioaccessibility of contaminants and nutrients, the properties, behaviors and fate of NNPs are not well understood. Advances in nanotechnology have enhanced our ability to measure these properties, yet much of this research focuses on engineered nanoparticles (ENPs) with homogeneous compositions, designed for use as uniform systems.[15] Due to the diversity and polydispersity of natural particles, tools developed for ENPs have thus far provided limited insights into the complex properties of NNPs. Diverse populations of NNPs comprise natural particle systems (NPS), which have not been adequately characterized due both to their diversity and the limitations of conventional mathematical models.[16] Given the nonadditive behavior of particles <50 nm and the great diversity of NPS, it is suggested that these systems should be treated as strongly correlated particle systems interacting across multiple scales.[17] This requires the integration of extensive property measurements, merging new nanoanalysis tools with advanced modelling and simulation methods to develop theoretical frameworks that better explain the behaviors of natural particles in environmental systems.

A process-based approach for simultaneously assessing the cycling of ENPs and NNPs has been proposed in literature.[18,19] In the aquatic environment, NPs interact with different abiotic and biotic components independently of their origin.[20] They are transformed *via* various interconnected and dynamic processes, such as aggregation, sedimentation, dissolution, chemical and physical alterations, and adsorption of pollutants and nutrients, which in turn control their transport, dispersion, biological accumulation, and biomagnification, and thus ultimately govern their environmental impact (Fig. 1).[21–23] The nanometre size, structural heterogeneity, and low

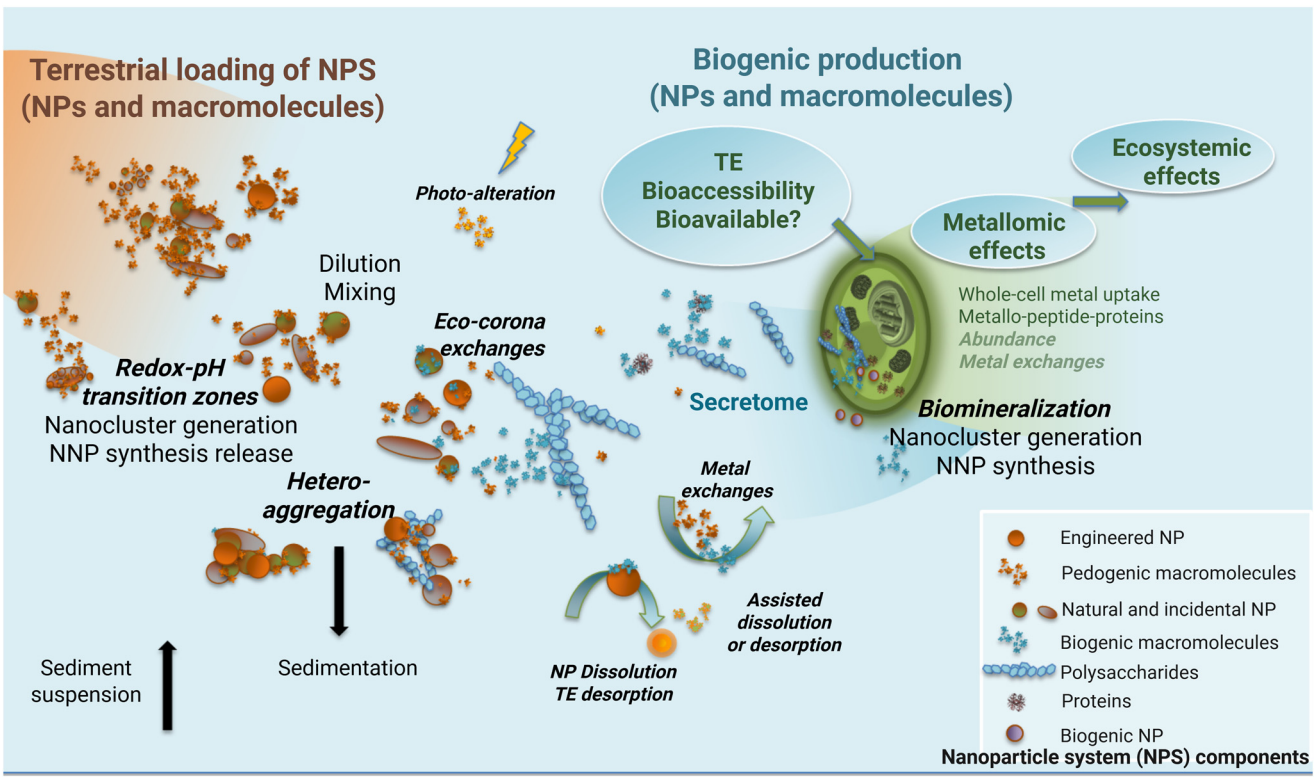


Fig. 1 A visual representation of the primary components of nanoparticle systems (NPS) and the compartments and processes associated with environmental nanobiogeochemistry. The focus is on metal-based nanoparticles (NP), macromolecules, their hetero-aggregates and microorganisms as it is the "size-range" of analytes that can be measured by AF4-ICP-MS and spICP-(TOF)MS. Hydrated ions, small molecules, and small complexes were intentionally excluded.

concentrations of ENPs, combined with the dynamic nature of their transformations, make their quantification in the aquatic environment very challenging. However, developments in nanoanalysis driven mainly by the desire to characterize and tailor the properties of ENPs, and concerns regarding their fate and effects in natural systems, have opened novel avenues towards understanding NP cycling on Earth.[24] The advantages and limitations of different analytical approaches proposed for the characterization and quantification of ENPs in the environment have been comprehensively reviewed.[25–27]

Among different complementary approaches such as microscopic, spectroscopic, mass spectrometric, electrochemical, and size-fractionation methods, inductively coupled plasma mass spectrometry (ICP-MS) –based methods have demonstrated outstanding capabilities for determining and characterizing nanomaterials in complex environmental and biological settings. This includes single particle ICP-MS (*i.e.*, spICP-MS) alone and ICP-MS in combination with size-based separation techniques such as asymmetric flow field-flow fractionation (AF4). Electron microscopy techniques such as TEM and related synchrotron-based techniques will remain important tools for high-resolution analysis; however, they are low-throughput techniques that are prone to artefacts during sample preparation. Approaches using spICP-MS and AF4-ICP-MS overcome these shortcomings, making their combination a promising approach for obtaining multifaceted information about the concentrations and distributions of the sizes and elemental compositions of NNPs that comprise an NPS with minimal disturbance at environmentally realistic concentrations.[24,27]

This first section of a two-part review explores the fundamentals, advantages, and disadvantages of these methods in environmental nanobiogeochemistry. The companion review, Exploring environmental nanobiogeochemistry using field-flow fractionation and ICP-MS-based tools: progress and frontiers, reviews recent applications of these tools in environmental nanobiogeochemistry, combining published and unpublished data in case studies to delineate frontiers and identify needs for future research.

2. What is environmental nanobiogeochemistry?

Nanogeochemistry is the study of naturally occurring materials and processes at the nanoscale.[1,9] Environmental nanobiogeochemistry focuses on the environmental geochemistry of NNPs and NPS, including their ecosystem functions and interactions with organisms, and quantifying the impacts of disturbances thereupon. In a more integrative form, this is the study of the Earth as a chemical system at the nanoscale, exploring the dynamic interactions of elements and compounds with biological and geological components and their possible alteration by human activities.

NNPs comprise the vast majority of NPs in the environment and are formed *via* numerous abiotic and biological processes in both top-down (erosion, weathering) and bottom-up (neoformation, precipitation) schemes (Fig. 1).[1,28] Inorganic NNPs can be divided into two broad classes: 1) nanominerals that have crystal phases and stoichiometries that only exist at the nanoscale and 2) mineral nanoparticles that can be in the nano-, micro-, and bulk (>1000 μm) size regimes.[9] Macromolecules, other forms of natural organic matter, and organic–inorganic aggregates are also nanoscale particles that play key roles in natural waters.[10] Regardless of their origin and composition, their small size, high reactivity, and unique properties play important roles in the transport of contaminants and the cycling of critical elements and nutrients such as Fe and P.[29–31] Despite their essential role in environmental processes, the ability to study their behavior, characterize their composition, and quantify their concentration is only now being realized through advances in environmental nanoanalysis.[24,32]

Understanding environmental nanobiogeochemistry requires a multidisciplinary approach. Geomorphology, geochemistry, and mineralogy provide an understanding of the source and transport of NNPs from the bulk material (*i.e.*, rock, soil, or sediment).[33–36] Hydrology[37,38] and oceanography[39,40] enable the study of material flows in the likely aqueous transport of these materials. At the atomic and molecular levels, biology, chemistry, and physics help to illuminate the synthetic routes and behavior of these materials,[41–43] while ecotoxicology plays an important role in determining their hazards and risks.[44,45] Furthermore, data scientists[46,47] and metrologists[27] create new opportunities and advance the scope of what can be studied. Though these examples are not exhaustive or complete in the roles various disciplines play in environmental nanobiogeochemistry, they highlight the need for both a multidisciplinary approach and collaborative problem-solving approaches.

Developing a more comprehensive and in-depth understanding of environmental nanobiogeochemistry is increasingly urgent as the Earth undergoes dramatic transformations driven by anthropogenically-induced changes, including urban development, technological advances, and climate change. Increasing urbanization has led to increased inputs of anthropogenic (*i.e.*, ENPs) and incidental NPs (INPs) into the atmosphere and aqueous environments, though their levels remain significantly lower than those of NNPs.[48,49] While ENPs such as silver NPs are intentionally manufactured for some purpose, INPs such as brake dust are created incidentally due to other activities[1] or by weathering of the original material, such as plastic based products.[50] These ENPs and INPs have various compositions ranging from brake dust resembling NNPs (*e.g.*, magnetite (Fe_3O_4))[51,52] to particles with engineered structures and compositions (*e.g.*, quantum dots[53] and nanoplastics[54]). Their interactions with NNPs and living organisms are poorly understood, and the lack of categorization of different NPs populations as NPS, in addition to their discrete and

nonadditive behaviors,[17] can complicate our understanding of nanomaterial and biological processes.

Recent technological developments in the fields of renewable energy, medicine, and agriculture may also serve as point and non-point sources of NP emissions to the environment.[55,56] The global transition to low-carbon forms of energy including solar, wind, nuclear, and battery technology has led to extensive mining operations, which can increase the fluxes of these materials into environmental compartments.[57,58] Upon use and disposal, and in the absence of recycling, these materials may also find entry into the environment *via* improper disposal or leaching from landfill and e-waste sites.[59,60] Similarly, as the use of nano-pesticides and nano-fertilizers becomes more prevalent, unintended run-off into streams and waterways may lead to a considerable input of ENPs and constituents that can transport and interact with pre-existing NNP populations.[61–63] While these ENPs, INPs and other NPs such as quantum dots and nanoplastics constitute disturbances to the natural nanoenvironment, the range and functioning of NNPs and NPS, and disturbances thereto, are the primary focus of environmental nanobiogeochemistry—since ENPs and INPs make a relatively small contribution to most NPS,[1] they are only indirect foci of study within this scope.

It is with climate change that we may see the most dramatic change in NNP and NPS dynamics, as the very source of elemental feedstock into waterways can be radically altered by various climatological effects.[64] Accelerating glacial melt can liberate previously frozen sources of Fe particulates, serving as a new source of this nutrient limiting element in estuaries.[65–67] The browning of boreal lakes and rivers also provides additional Fe to estuaries with enhanced transport to oceans due to their robust organic matter coronas, potentially causing harmful algal blooms while increasing carbon sequestration on the ocean floor.[68–72] Desertification and anthropogenic land use changes will increase fluxes of wind-blown dust, altering both solar radiative forcing and global nutrient cycling.[73] Permafrost undergoing thaw can release additional colloidal (1–1000 nm) and nanoparticulate (1–100 nm) materials, often along with other toxic elements such as As and Hg, which can further be transported *via* colloids and nanovectors.[74–77] Previously predictable weather patterns now give way to extreme drought, exposing sulfidic minerals in sediment and potentially leading to the release of metals and acidity.[78,79] Just as we are beginning to be able to characterize and quantify NNP populations, the number and composition of these materials are poised to undergo significant changes necessitating comprehensive study to better comprehend the impacts of climate change.

From these examples we note the vast complexity of nanomaterial compositions and populations present in environmental systems. With myriad types, sizes, and shapes, it is expected that the interactions between NPs and other environmental constituents (*i.e.*, solutes, interfaces, biota) are dynamic and interconnected. Consequently, attempting to derive particle behavior from any one single measurand will provide a decidedly incomplete and likely inaccurate representation of their properties and associated roles in the environment. For this reason, attempts to understand environmental nanobiogeochemical processes will require analytical approaches that provide not only a measure of key particle properties (*e.g.*, size, number, composition), but also their relationships with other particles and molecules (*e.g.*, aggregation state, surface coating), organisms, and their responses. Advanced analytical methods using techniques such as FFF and ICP-MS provide a more comprehensive picture of these interactions with a focus on distributions, and continued developments will lead to a more accurate understanding of environmental nano-interactions.

3. Fundamentals of FFF and ICP-MS

Field-flow fractionation (FFF), particularly asymmetric flow FFF (AF4), is a key method for separating and analyzing NPS across a size continuum.[80–82] This technique uses a minimally disruptive, diffusion-based separation which can be coupled with various detectors (*e.g.*, UV-visible, fluorescence), making it ideal for characterizing macromolecules, nanoparticles and microparticles. Diffusion-based separation and the corresponding lack of a stationary phase in FFF makes it distinct from chromatography, where adsorption to or size exclusion from the stationary phase are the primary separation mechanisms. This means that the elution order is also reversed compared to chromatography in size-based FFF techniques like normal/Brownian mode AF4, such that smaller particles exit the channel before larger particles. The lack of stationary phase also eliminates interactions with the analyte which lead to elevated shear forces. Minimizing shear forces and adsorption are important for preserving natural conditions, which is critical for the analysis of NPS, since high shear forces destroy weakly connected aggregates. Interactions with a stationary phase also lead to the loss of smaller molecules and particles through adsorption.[83]

Field-flow fractionation can also be coupled to multiangle laser light scattering (MALS) and dynamic light scattering (DLS) detectors ideal for characterizing the size of NNPs,[84–87] making the overall techniques suitable for in-depth assessments of the size distribution of NPS and their components. Most importantly in the context of environmental nanobiogeochemistry, AF4 can also be paired with ICP-MS for comprehensive analysis of both trace elements, including contaminants and nutrients (*e.g.*, Pb, Tl, P), and major components of transport vectors (*e.g.*, Al, Fe, Mn, Si).[88–104] Advances in ICP-MS, especially with single-particle (sp)ICP-MS and spICP-time-of-flight (TOF) MS, allow detailed elemental analysis of individual particles, further enhancing the study of NPS.[102,105–112] Below, the relevant fundamentals of FFF and ICP-MS are reviewed, including their coupling and operating ICP-MS in single-particle mode.

3.1. FFF: fundamentals and fields

Field-flow fractionation (FFF) originated in the mid-1960s with Giddings' concept.[80,113] The key principle of FFF is the application of an external field perpendicular to the laminar flow of a carrier fluid in a thin, ribbon-like channel. This field causes the sample components to diffuse into size-dependent flow lamina so that they migrate down the channel at different velocities, resulting in spatial separation. Due to the lack of a stationary phase and complex adsorption–desorption processes, the theory of FFF relating retention time and diffusion coefficients to hydrodynamic diameter are well defined for spherical particles with uniform density; however, most NNPs do not meet these criteria, so frequent channel calibration or verification of size using additional detectors such as MALS or DLS is required.[81,87] The separated components then elute from the channel at different times, allowing collection and analysis. While FFF gained experimental realization in the late 1960s, it has since evolved with different modes and found applications across diverse fields; it is now an advanced and widespread separation technique used to separate particles and macromolecules based on their size, shape, and density.[114–116] Different types of external fields may be applied to induce differential migration and separation of sample components within a channel based on the properties of interest (Table 1).

Gravitational or split-flow thin (Gr or SPLITT) FFF utilizes the force of gravity to separate particles, with larger and denser particles sedimenting faster and being retained longer. GrFFF is useful for performing non-destructive binary separation of larger particles, such as micrometer-sized colloids[117] and biological cells.[118] This process is a useful alternative to other separative techniques with known artefacts, such as filtration and centrifugation. Its setup and operation are simple, but it is limited to particles that experience sufficient gravitational force and so is typically not effective for separating particles with sizes in the nanoscale range.

Sedimentation/centrifugal (Sed) FFF employs a centrifugal field to separate particles based on their buoyant mass.[119] Heavier and larger particles sediment faster and are retained longer in the channel. SedFFF is effective for separating large particles such as cells, organelles, and micron-sized colloids.[120] This technique is suitable for a wide range of particle sizes, including very large particles, but the centrifugal forces can affect delicate samples and require specialized equipment. The centrifugal field is also limited to size-based separation for particles with diameters $>$ *ca.* 10 nm.

Thermal (Th) FFF uses a temperature gradient to induce differential migration based on thermal diffusivity.[121] ThFFF is particularly useful for separating polymers, proteins, and other macromolecules with subtle differences in size and composition. This method provides high resolution for particles with small size differences but may affect temperature-sensitive samples and requires precise control of temperature.

Electrical field-flow fractionation (ElFFF) employs an electric field to separate particles based on their charge and size.[122] ElFFF is ideal for separating charged particles such as proteins and nanoparticles. This technique can simultaneously separate particles based on size and charge, but it is limited to charged particles and requires careful control of the electric field strength to avoid damaging the sample.

Flow field-flow fractionation (FlFFF) uses a liquid flow perpendicular to the main channel flow to separate particles (Fig. 2).[123] The separation process involves two major steps: injection/focusing and elution. There are two opposing flows in the focusing stage, allowing NNPs to enter the channel through the tip flow during injection and move to the focusing position where the tip flow is met by the opposing focus flow. The sample then forms a tight band at one location in the channel, where the crossflow induces diffusion away from the membrane that is proportional to the hydrodynamic size of particles in accordance with the Einstein–Stokes relationship.[81] Once the positions of the particles have equilibrated, the focus flow is stopped and the same flow rate is added to the other flow entering the channel, so that the particles move down and out of the channel in the elution step, whilst the crossflow is maintained. In Brownian/normal mode, smaller particles elute earlier, while larger particles are retained longer due to their slower migration. In steric mode, large particles are subject to hydrodynamic lift forces that cause them to migrate away from the walls and towards the center of the

Table 1 Types of field-flow fractionation and their common uses

FFF type	Field	Separation variable	Common applications
Flow	Liquid flow	Hydrodynamic size	Colloids, NNPs, ENPs, macromolecules, polymers, liposomes
Gravitational/SPLITT	Gravity	Size/density	Cells, polymers, microorganisms, sediment/soil particles
Centrifugal/sedimentation	Centrifugal force	Buoyant mass	Colloids, NNPs, ENPs, cells, macromolecules
Thermal	Temperature	Molecular mass, chemical composition	Polymers, gels, macromolecules
Electrical	Electric field	Electrophoretic mobility, size	Cells, colloids, organelles, macromolecules, latexes
Hollow fiber flow	Liquid flow	Hydrodynamic size	ENPs, liposomes, polymers, gels, macromolecules
Magnetic	Magnetic field	Magnetic moment, size	Magnetic ENPs, NNPs and microparticles

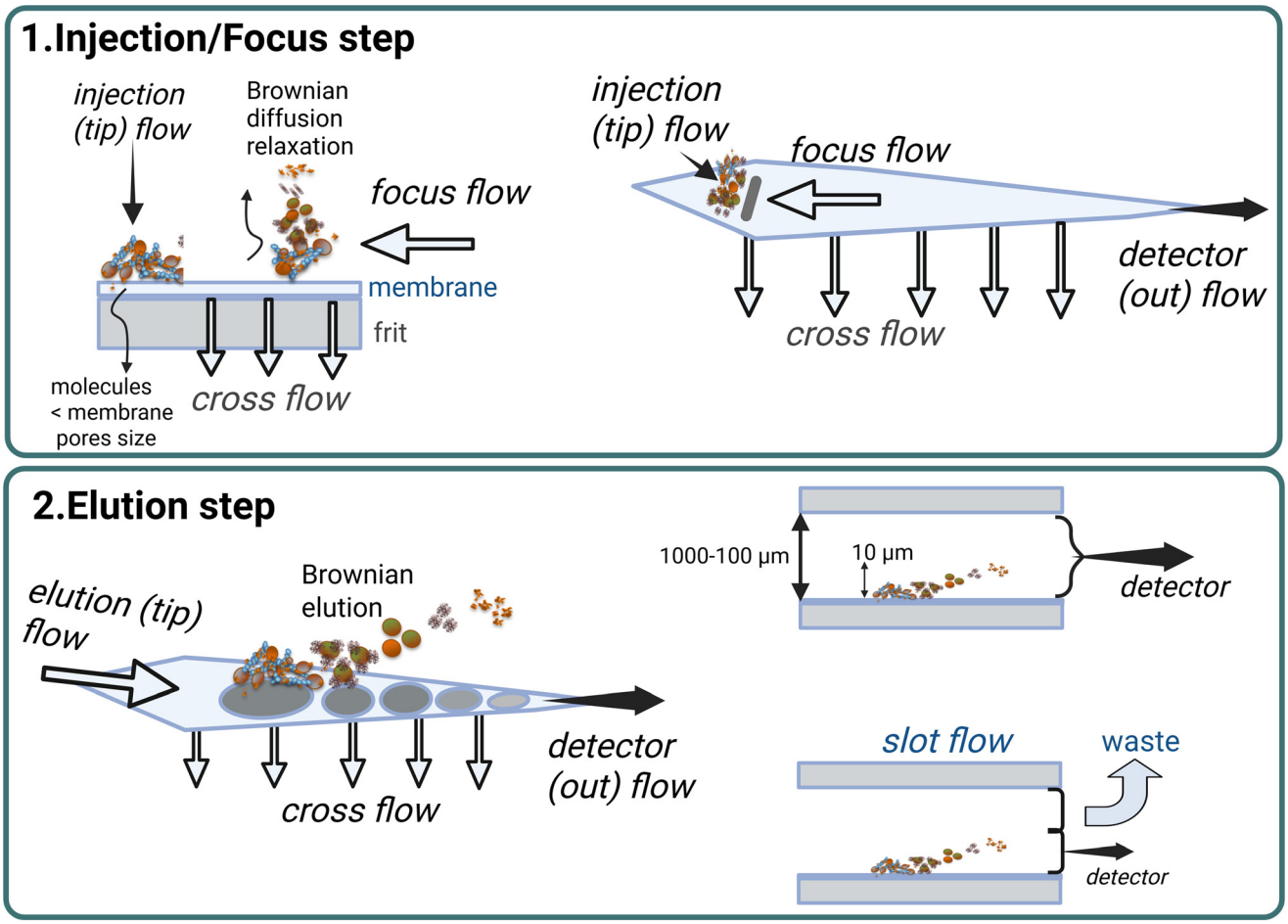


Fig. 2 Schematic of major separation processes and fluxes operations associated with asymmetric flow field-flow fractionation.

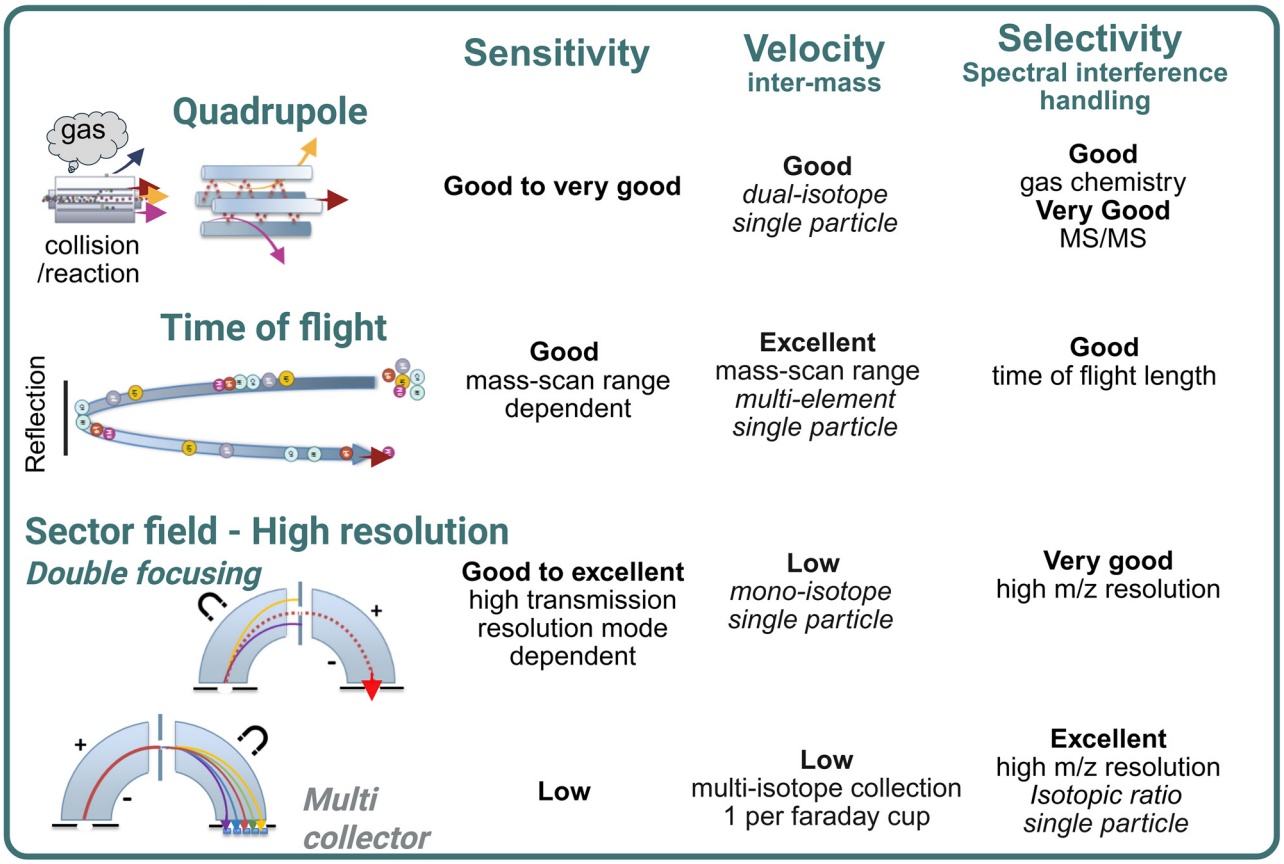
channel. Since larger particles have a larger surface area, they are more influenced by the flow of eluent down the channel and they elute before smaller particles, which are more influenced by diffusion and tend to stay closer to the walls.[124] The expected size-based elution order is thus inverted due to this 'steric inversion' effect. Flow FFF is ideal for separating macromolecules and colloidal particles with diameters in the range of a few nanometers in various environmental and biological samples. Its high resolution and gentle separation conditions preserve the integrity of fragile samples, although it requires careful control of flow rates to optimize resolution within the size range of interest. Due to its versatility and ability to separate the smallest nanoparticles by size, flow FFF, especially asymmetric flow FFF (AF4), is the favored FFF technique for environmental analysis and coupling to ICP-MS. ElFFF and AF4 have recently been combined in EAF4 instruments, providing separation using either one or both fields, with the added advantage of measuring zeta potential.[125]

Overall, FFF techniques offer versatile and high-resolution separation capabilities for a wide range of applications in environmental nanobiogeochemistry. By selecting the appropriate field and optimizing operational parameters, FFF can effectively separate complex mixtures of particles and macromolecules, enabling detailed analysis of their composition and behavior in various environmental contexts.[87,114]

3.2. ICP-MS: fundamentals and mass analyzers

Inductively coupled plasma mass spectrometry (ICP-MS) has been a pivotal analytical technique since its introduction in the early 1980s.[126] Originally developed for atomic emission spectroscopy, the coupling of the ICP source with mass spectrometry has significantly improved the determination of trace metals and other elements.[127] The low detection limits and multi-element capabilities of ICP-MS make it indispensable for trace element (TE) determination in environmental matrices such as water, soil, and biological samples. Its robustness allows it to handle complex solutions, facilitating the direct determination of elemental concentrations in complex matrices with a linear response over several orders of magnitude, despite potential interferences.

In conventional mode ICP-MS, the sample is nebulized and introduced into a plasma source where droplets are



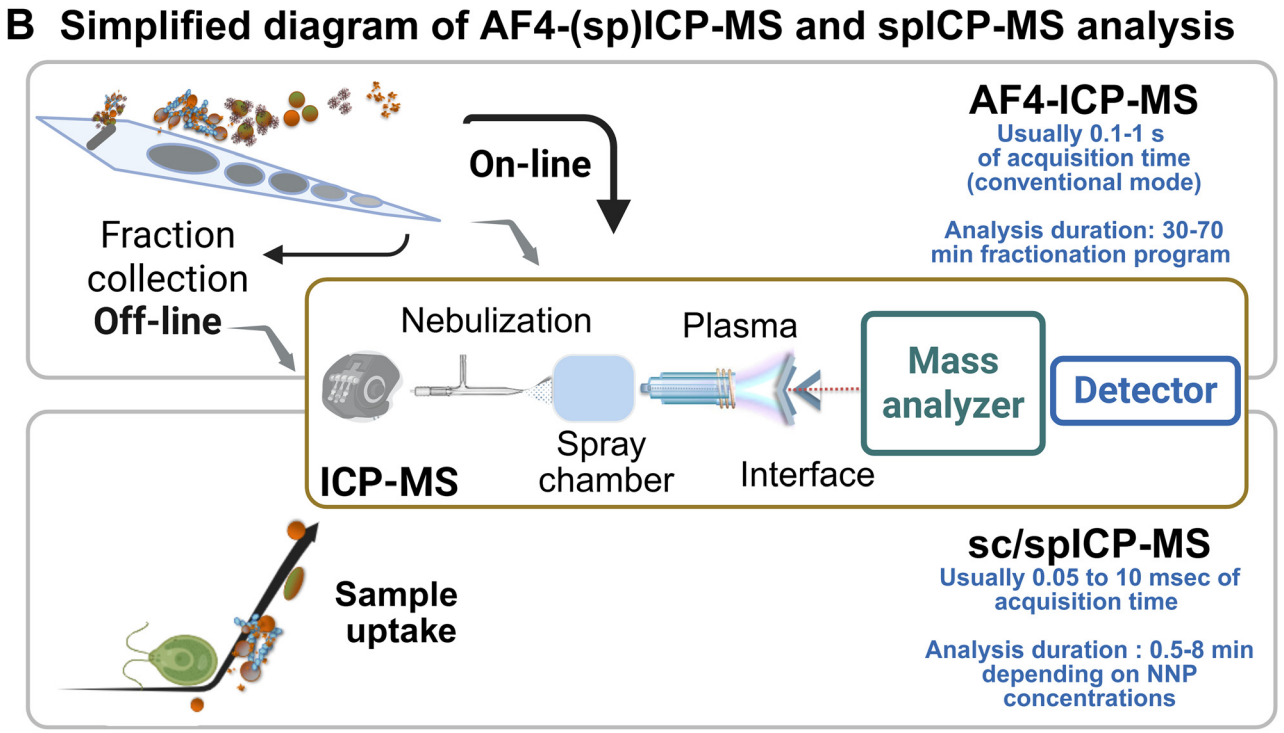


Fig. 3 Most common types of ICP-mass analyzers (A) used for coupling with AF4 and for spICP-MS and single-cell (sc)ICP-MS analysis of NPS, as schematized in (B). (sp)ICP-MS: AF4 can be coupled on-line with ICP-MS in the normal or spICP-MS modes without or with fraction collections, as indicated with grey arrows. The capabilities of each mass analyzer in terms of acquisition time frequency and inter-mass velocity, as well as the individual component assemblages for quadrupole interference removal depend on the manufacturers. Note that dual-isotope measurements for the latter mass analyzers are not provided as a quantitative option commercially. See the literature for more information.[133–135]

desolvated and elements are vaporized, atomized and ionized.[127] The plasma gas is sampled through the plasma-mass spectrometer interface, and the ions are separated from the neutrals and focused in the ionic lenses, allowing the ions to be directed into a mass spectrometer (*i.e.*, mass analyzer described below and Fig. 3), which separates based on mass-to-charge ratio (m/z). The separated ions are then detected, with ion counts proportional to the quantity of the targeted element, allowing for both qualitative and quantitative analysis.[128]

Interferences in ICP-MS measurements represent a challenge for measuring element concentrations, potentially affecting the accuracy of analysis.[129] There are different categories of interferences: spectral or non-spectral. Briefly, spectral interferences derive from the overlap of signals of isobaric species (*i.e.*, $^{40}Ar^{+}$ interfering with $^{40}Ca^{+}$), polyatomic ions (*i.e.*, $^{40}Ar^{16}O^{+}$ interfering with $^{56}Fe^{+}$), or doubly charged species (*i.e.*, $^{138}Ba^{2+}$ interfering with $^{69}Ga^{+}$).[130] Non-spectral interferences include matrix effects and signal loss from deposits on the interface.[131] Addressing interferences using solutions such as a reaction/collision cell, an internal standard, and the optimization of sample preparation are key to ensuring accurate ICP-MS measurements.[132]

Mass analyzers separate ions based on their mass-to-charge ratio using various types of devices such as quadrupole (Q-MS),[136] tandem-mass spectrometry,[137] time-of-flight (TOFMS),[138] and sector field double-focusing/high-resolution (HR-MS) mass spectrometers.[139] Each analyzer offers different advantages in terms of simplicity, resolution, sensitivity, and interference removal for accurate quantification in environmental matrices (Fig. 3B). For example, a Q-MS consists of four parallel electrodes and operates by applying both direct current and radiofrequency potentials, creating an electrostatic field that filters ions based on m/z. While Q-MS systems offer sufficient resolving power (R = nominal mass/peak width at half maximum $\approx$ 300) or resolution ($\sim$1 amu) for most applications, they struggle with certain interferences, necessitating the use of collision cells to preferentially remove interfering ions or reaction cells to change the mass of the interfering ion or species of interest. With similar resolving power to a Q-MS, tandem MS systems facilitate improved analysis in the presence of interferences by using two quadrupoles separated by a reaction cell. This setup allows for selective removal of interferences through differences in reaction kinetics or products, significantly improving sensitivity and accuracy. However, the complexity and cost of ICP-tandem MS systems are higher compared to Q-MS systems. Combining a magnetic sector with an electrostatic analyzer (*i.e.*, double-focusing), HR-MS offers superior resolving power ($R \geq 10\,000$) essential for deciphering complex interferences. Double focusing ICP-MS instruments can be equipped with a single detector (single collector, SC) or multiple detectors in an array designed to simultaneously record signals from multiple isotopes (multicollector, MC). The simultaneous detection capability provided by MC-ICP-MS is essential for monitoring fast transient signals for multiple isotopes, including single particle analysis. However, the high cost and complexity of HR-ICP-MS require skilled operation and maintenance, which can be a barrier for some laboratories.

The TOFMS separates ions of different masses based on differences in their flight time, offering rapid and pseudo-simultaneous detection of multiple elements. This high-speed analysis is ideal for capturing high frequency transient signals but generally provides lower sensitivity and resolving power compared to other mass spectrometers (*ca.* $850 \leq R \leq 6000$, depending on the model). The addition of a reflectron improves resolution by doubling the ion travel distance (U-shaped path) and reducing the width of the kinetic energy distribution.

These various mass spectrometer types, with their ability to analyze TE in various environmental matrices from water and soil to biological samples, enable researchers to gain valuable insights into the dissemination, behavior, and impact of trace elements and nanoparticles in the environment.[133,134,140–142] Overall, ICP-MS-based techniques play a crucial role in advancing our understanding of the environmental processes governing the distribution of TE in environmental systems.

3.3. Single-particle ICP-MS (spICP-MS)

The development of single-particle ICP-MS (spICP-MS) marked a significant advancement in the characterization of nanoparticles.[143] Its history is closely tied to the development of plasma sources and the need to characterize individual aerosol particles in the atmospheric sciences. In 1986, Kawaguchi *et al.* used a plasma source to excite micrometer-sized particles generated from monodisperse droplets of inorganic compounds. Signals were recorded using a digital oscilloscope at a frequency of 1 kHz to distinguish individual particles.[144] Later studies in the late 1980s and 1990s demonstrated that the particle size distribution could be determined using ICP-OES, and further improvements in ICP-MS detection limits enabled the characterization of elements in aerosols at femtogram-level concentrations.[145–148]

The application of spICP-MS to colloidal suspensions and NNPs in the early 2000s laid the groundwork for current methodologies.[149–152] For conventional analysis, acquisition times typically range from 0.1 to 1 s, resulting in steady signals, while, for single particle mode, higher time resolution with low acquisition (dwell)-times is required for event detection, typically ranging from 0.05 to 10 ms (Fig. 3B). This approach involves nebulizing a suspension of nanoparticles into the plasma, where each particle undergoes the same ionization process described above. The resulting ion cloud is detected with high temporal frequency, allowing for the differentiation of nanoparticles from the background signal. The number and intensity of events respectively correlate with the number of nanoparticles in the sample and the amount of the

measured isotope therein, providing quantitative data on particle concentration and mass/size distribution.[143,153] It is particularly important to pay attention to certain parameters to avoid the joint arrival of NPs (>one NP per dwell-time). This would distort the result by suggesting a lower number of NPs with a higher average intensity, consequently underestimating the concentration in number and overestimating the diameter/mass. Laborda *et al.* estimated this coincidence probability (joint arrival of NPs) using a Poisson distribution, enabling the analysis to be carried out at higher NP number concentrations.[154]

Individual NP events typically require 200–1100 μs for the ion cloud to pass the detector, presenting a significant barrier to the simultaneous monitoring of multiple elements/isotopes necessary for a proper understanding of nanoparticle biogeochemistry.[143,155] While the traditional single-particle mode using sector field mass spectrometry (HR-MS) offers low size-based detection limits (diameters ≤10 nm for some elements),[156–158] challenges arise due to the slow rate of changing the magnetic field, hampering the simultaneous monitoring of multiple elements within a single NP. This challenge can be overcome by using a multicollector (MC-ICP-MS), which allows for precise elemental and isotopic ratios to be measured in NPs.[159–161] However, dwell times are limited to 25–50 ms, requiring extremely dilute suspensions and long analysis times. More recent developments in quadrupole mass spectrometry (Q-MS) are promising, with manufacturers improving the time required to switch between measuring different masses and acquisition rates ≤100 μs, thereby improving the temporal resolution for single particle analysis. Although settling times between measurements are relatively short (~100 to 500 μs), allowing monitoring of multiple elements in each NP event, the technique is currently limited to one or two targeted elements.[162] This remains the case when using more recent instrumentation that includes the collision cell, which enlarges the span of ion clouds in particle events.[163] An exhaustive list of settling and dwell times for various manufacturers and models is beyond the scope of this review, but it is worth noting that they differ significantly and should be a primary consideration when selecting an instrument for single particle analysis.[135]

The shortened spectral acquisition time highlights a significant advantage of the TOF mass analyzer for single-particle analysis, allowing pseudo-simultaneous monitoring of all m/z in the mass spectrum every 25–30 μs.[164] Thus, an ICP-TOFMS near-continuously collects the multi-ion beam at a rate of approximately 25–30 μs per spectrum.[165] This feature improves the temporal resolution of NP signals while ensuring the measurement of many elements in all NPs. Consequently, the identification and classification of ENPs and NNP and their roles in complex natural matrices (*e.g.*, environmental, geological, biological) benefits significantly from the depth of information available by applying ICP-TOFMS to NNPs and NPS.[48,49,111,166,167]

3.4. FFF and online coupling with conventional mode ICP-MS

The combination of FFF with ICP-MS operating in conventional mode is a powerful hyphenated technique for the comprehensive analysis of complex environmental samples.[87,88,168–170] The separation capabilities of FFF allow for size-based fractionation of particles and macromolecules based on their physical and chemical properties, while ICP-MS provides highly sensitive and selective elemental detection and quantification. In this coupled setup, the eluent from the FFF channel is directly introduced into the ICP-MS for real-time characterization of nanoparticles, colloids, and macromolecules, providing insights into their size distribution, elemental composition, and potential speciation. One of the main advantages of FFF-ICP-MS is its ability to handle complex samples with minimal sample preparation, preserving the integrity of fragile particles and reducing the risk of contamination.

The hyphenation of FFF with ICP-MS enhances the capabilities of both techniques. FFF separates the components based on their size, shape, or density, which resolves particle properties that may vary with size. It effectively lowers the dissolved background and reduces associated matrix effects when simple complexes and small molecules pass through the separation membrane and adequate carrier fluid is used. Real-time elemental analysis by ICP-MS allows for the identification and quantification of trace elements in each size fraction and corresponding type of nanoparticle/colloid (*e.g.*, small complexes and molecules, elements associated with organic matter, and primarily inorganic particles), providing valuable information on the distribution and concentration of elements within different size ranges. With proper optimization, FFF-ICP-MS offers a robust and versatile platform for environmental nanobiogeochemistry, enabling detailed investigations of the behavior, fate, and impact of NNPs and other particulate matter in various environmental matrices.

3.5. Coupling of AF4 and sc/spICP-TOFMS

The AF4 system is particularly advantageous for separating a wide range of particle sizes, from nanometers to micrometers.[81] Compared to other FFF-based techniques, the trapezoidal channel used in AF4 improves resolution and separation efficiency for polydisperse samples. Coupling AF4 with spICP-MS or spICP-TOFMS allows the detailed characterization of size-resolved particles. In an off-line setup, fractions collected from AF4 are introduced into the ICP-MS or ICP-TOFMS operating in sp-mode for elemental analysis. This method provides several benefits: it avoids potential issues related to flow rate compatibility and interface optimization between AF4 and ICP-MS (as discussed in the following section), allows for the analysis of complex samples that may otherwise clog or damage the ICP-MS system, and enables the use of different analytical techniques on the same fraction while decreasing the ICP-TOFMS running time, since an optimized AF4 separation can take

several tens of minutes. This off-line hyphenation of AF4's high-resolution separation and spICP-TOFMS's sensitive, high-throughput elemental analysis at the single particle scale thus offers a powerful approach for studying the size, composition, and distribution of nanoparticles in environmental samples. AF4 may also be coupled to single-cell (sc-) or spICP-TOFMS online, with outflow from the AF4 flowing directly into the TOF instrument[109,171] The sc-mode of ICP-MS operates similarly to the sp-mode, with samples diluted so that the content of single cells are ionized, and typically employs a specialized nebulizer to transport cells to the plasma. While coupling AF4 online to ICP-MS operating in sp- or sc-mode has significant potential, it has been primarily demonstrated only in proof-of-concept studies.[172]

4. Optimization, advantages and limitations

4.1. Optimization

4.1.1. Asymmetric flow field-flow fractionation. Method optimization plays a crucial role in ensuring effective separation and minimizing sample perturbation in AF4. Separation can be affected by various factors, including the aggregation of particles, interactions between particles and the membrane, premature elution caused by sample overloading or electrostatic repulsion between particles, and steric inversion. Steric inversion reverses the order of elution for particles with sizes > *ca.* 700–900 nm depending on the crossflow rate, so that larger particles elute before smaller particles, requiring the separation of small and large particles prior to analysis. Other key considerations discussed below include choice of carrier solution, membrane type, applied field and flow program, and sample loop size.[86,87,173–175]

The carrier solution should match the sample's physicochemical properties, such as pH and ionic strength, to minimize disruptions to NNPs. Maintaining these properties near to *in situ* conditions is crucial because pH and redox potential significantly influence metal speciation and the reactivity of metal-binding functional groups. Significant deviation from natural conditions will therefore lead to an inaccurate representation of the TE distributions among various colloid and nanoparticle populations due to changes in surface charge, double layer thickness, particle aggregation or disaggregation, and dissolution.[86]

Carrier solutions typically include monovalent ions such as $NaNO_3$, CH_3COONa, NH_4NO_3, and CH_3COONH_4 because they do not participate in complexation or precipitation reactions with the particles. Divalent ions such as $(NH_4)_2CO_3$ may also be used when the associated elements do not cause interferences. However, a carrier solution like NaCl can also enhance polyatomic interferences in ICP-MS measurements due to abundant chloride isotopes, and $^{23}Na + {}^{40}Ar$ may also serve as an interferent for ^{63}Cu when any Na-containing compound is used. The quantification of associated TE also differed when comparing 10 mM NH_4NO_3 and 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonate sodium (HEPES) for measuring the association of Hg to an organic matter standard.[104] Typically, the constituents of carrier solutions are added at concentrations in the range of 0.1 to 10 mM to minimize membrane interactions, but higher concentrations may also be needed to match the conditions of the sample matrix. The carrier solution must also work well with system components like tubing, pumps, and valves while meeting the needs of any coupled detectors. The characteristics of the carrier fluid can also affect the recovery of latex nanoparticles and NNPs.[176,177] Notably, NaCl is widely used at low concentration for the determination of size-based composition of NNPs;[178] a collision cell is helpful for removing polyatomic interferences at these low concentrations.

To ensure optimal recovery and minimize losses, the membrane which comprises the accumulation wall should be chosen carefully. It should effectively retain the maximum number of NNPs within the channel while reducing interactions between the particles and the membrane (Fig. 4). There are various membrane materials available, such as regenerated cellulose, cellulose triacetate, poly(ethersulfone) (PES), polypropylene, polyamide, polycarbonate, and polyvinylidene difluoride.[179,180] These materials vary in factors like thickness, surface characteristics, surface charge, smoothness, and both mechanical and chemical stability. Separation membranes commonly have pore sizes of 0.3, 1, 5, 10, 30, and 150 kDa. Smaller pore sizes help retain both smaller particles and charged species such as molybdates and vanadates.[100] However, membranes with smaller pores limit the range of separation conditions because they limit the flow rate of water through the pores (*i.e.*, cross-flow rate or field strength). Smaller pore sizes require a small inner diameter of tubing exiting the channel to force flow through the membrane at the desired rates, which increases pressure within the system and can lead to damage or leaking if care is not taken to monitor and limit system pressure.

The membrane pore size should be carefully selected when analyzing acidic and DOM-rich waters.[180] For instance, 0.3 and 1 kDa PES membranes were unsuitable for separating colloids and associated TEs in acidic, DOM-rich peat bog porewaters due to rapid membrane clogging.[181] A 5 kDa PES membrane was thus recommended for peat pore waters, while a 1 kDa PES membrane was more appropriate for moss waters.

In general, membrane cutoffs and the "sizes" of organic NPs are expressed in terms of Da, which is a unit of molecular mass and not size. This is because the sizes of these pores and organic NPs like macromolecules, proteins and organic aggregates are dynamic, with conformation and associated size depending on solvent–macromolecule interactions and associated factors such as pH, ionic strength, and concentration. However, if a roughly spherical conformation is assumed, then the relationship between size and molecular mass can be estimated using the relationship R (in nm) = $(3V/4\pi)^{1/3}$ = 0.066 × $M^{1/3}$ for volume V and molecular mass M (Da).[182]

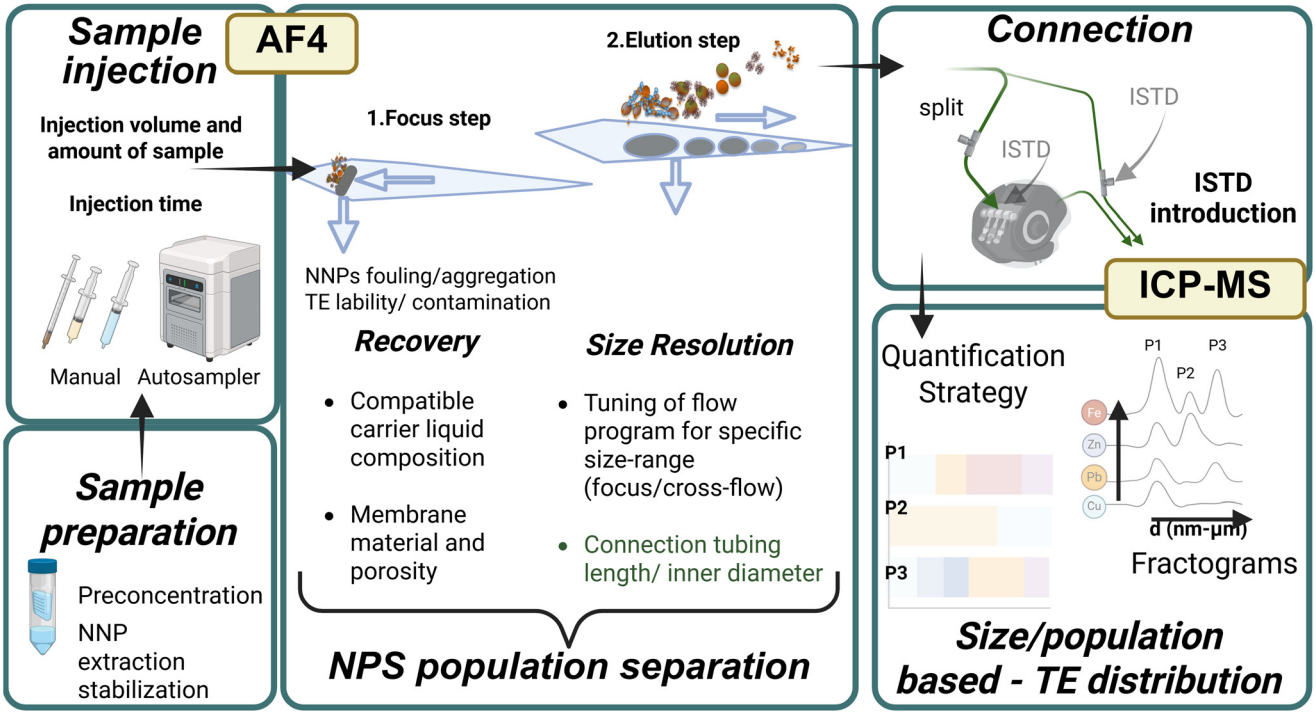


Fig. 4 Schematic workflow of the optimization of parameters in AF4 coupled with ICP-MS to obtain proper separation and analysis of TEs associated with NNPs. ISTD: internal standard.

To reduce particle–membrane interactions and improve sample recovery, the membrane charge should be as close as possible to the charge of particle surfaces.[174] However, the ubiquitous presence of polyfunctional, polyelectrolytic organic matter and particles with various surface properties in NPS makes exact charge-matching impossible. Interactions and significant adsorption leading to the loss of particles may also be minimized by incorporating surfactants into the carrier fluid, like Triton X-100, sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), or polysorbates such as Tween.[22] However, surfactants may also alter the electrical double layer and disperse aggregates in natural samples, such that the measured size distribution is no longer representative of natural conditions. Concentrations of trace elements in commercial surfactants may also be too high, serving to contaminate the relatively low concentrations in natural samples, particularly when those elements are then separated according to size.

The field strength in AF4 should be optimized to ensure the best possible separation while minimizing band broadening, sample losses and reducing overlap with the void peak.[100,175] Band broadening is controlled by minimizing the elution time, making it challenging to achieve high resolution across the broad size range of polydisperse NPS without flattening peaks; however, this can be improved using flow programming wherein the field strength is reduced with time so that larger particles are not retained in the channel for excessive periods. Adjusting the cross-flow rate to increase the resolution while maximizing recovery often requires optimization for different sample types. The cross-flow could thus also be adjusted to maximize resolution for the size range of interest. A high cross-flow is ideal for fractionating smaller NPS, while a lower cross-flow is more suitable for fractionating larger components of NPS.[175,183] For heterogeneous samples with a broad size distribution, a gradient cross-flow may be necessary to reduce analysis time and minimize steric inversion.[184] Steric inversion reverses the size-based elution order, leading to a non-linear relationship between retention time and size and mixed elution behavior of NNPs. It occurs for particles larger than *ca.* 700–900 nm depending on the cross-flow rate.[86] Therefore, prior filtration or removal separation of larger particles through other means is needed to prevent this phenomenon.

The sample loop size also impacts the flow program and optimal analysis conditions. A small sample loop introduces less material into the channel, which can make it more challenging to measure TE concentrations using ICP-MS; however, a small sample loop reduces focusing time, and hence interactions with the membrane, losses of charged ions and particles with sizes less than the membrane pore size, and the total analysis time. Conversely, a larger sample loop is better for low-concentration samples. Increased membrane interactions caused by higher concentrations and extended focusing times may also amplify the carryover effect and raise the background concentrations of TEs, thereby increasing the limit of detection.[181] A semi-preparative channel allows larger quantities of material to be injected before overloading occurs and has been useful in overcoming such limitations.[185]

Pre-concentration using ultrafiltration devices has also been applied to soft- and sea-water systems using AF4-UV-Fluo-ICP-MS, facilitating insights into the origin and dynamics of organic matter, together with size-based speciation of TE and associated NPs for aquatic systems with low NP counts.[186] However, systematic studies of the stability of NPs and TE speciation in this context are also needed for quantitative and comparable measurements following pre-concentration.

While pre-concentration methods like ultrafiltration and centrifugation offer the advantage of increased sample concentration, they come with drawbacks such as potential alteration of NPS, membrane interactions, and operational challenges such as memory effects and contamination resulting from inadequate cleaning and/or low concentrations in samples that are easily contaminated.[187] Using a slot-flow to avoid dilution in the AF4 channel could be an elegant approach to sustain the preconcentration that occurs during the focus/injection step with minimal risk of contamination. Since separation of nano-objects occurs within the first tens of micrometers above the accumulation membrane, splitting the outflow with a slot-flow allows the upper layer of the carrier fluid contained in the channel to be evacuated to waste without impairing the transport of separated nano-objects to the detectors, minimizing their dilution (Fig. 2). However, it has rarely been applied for NNP analysis.[188–190]

Overall, AF4 represents a versatile tool for separating and characterizing the size distribution of NNPs. Indeed, size calibration through AF4 allows the correlation of retention time with size. This is achieved by using different monodisperse standards of accurately known size (*e.g.*, polystyrene beads, Au or Ag nanoparticle standards, proteins, or polystyrene polymer standards) to create a calibration curve. This calibration curve serves as a reference for the size determination of unknown particles based on their retention time.[104,191] The ability of AF4 to assess particle size is enhanced by the variety of detectors that can be coupled to it to confirm the calibration curve, such as multi-angle light scattering (MALS) and dynamic light scattering (DLS). Indeed, these detectors allow the precise measurement of NNP size and, when combined with information about the major chromophoric and fluorophoric macromolecular components measured using the UV-vis and fluorescence detectors, provide a comprehensive understanding of the overall particle system.[192]

4.1.2. Coupling AF4 to ICP-MS. Coupling AF4 with ICP-MS for the analysis of NNPs is challenging due to the complex properties of NNPs, including their low concentration and their polyfunctional, polydisperse, and polyelectrolytic character. Frequent calibration of both the AF4 and ICP-MS is therefore essential for maintaining precision. To minimize contamination, it is crucial to use metal-free reagents and materials cleaned in acid baths and to prepare solutions in controlled environments such as class 100 HEPA-filtered air cabinets. The deposition of DOM, TEs, and NNPs on the membrane with associated sample loss can be mitigated through regular system conditioning and targeted cleaning procedures, though these methods must be carefully tailored to avoid damaging sensitive components like ceramic frits.[104,193,194] Detailed methods for calibration, conditioning, and cleaning are available in the literature.[100,104,181]

The total length, inner diameter, void volume, and number of changes in the inner diameter of tubing and connections between the AF4 system outlet and the ICP-MS inlet should be minimized to reduce band-broadening. A mixing tee can be used to introduce acids and internal standards into the outflow of the AF4 before it reaches the ICP-MS to reduce adsorption and carryover effects and account for instrument fluctuations. The capillary tubing and nebulizer of the ICP-MS should be selected to support the combined flow rates. Alternatively, flow can be split at an additional junction to reduce the flow rate entering the nebulizer as controlled by the peristaltic pump. However, splitting the flow decreases the sensitivity, as some sample is diverted. Incomplete mixing of the sample with the acid and internal standards may also occur if the splitting junction is not far enough downstream of the mixing tee. The acid concentration and capillary tubing length should be optimized to ensure complete mixing and acidification of the AF4 eluent and to thereby limit losses or exchanges due to ions being adsorbed to/desorbed from the tubing or other surfaces before it enters the spray chamber;[181] however, it is critical to ensure that high concentrations of acid are not introduced to the ICP-MS, as may happen when the AF4 channel is being rinsed, with the purge valve open. Some applications may not require acidification or internal standards if analyte concentrations are high enough that contamination or losses due to ad/desorption are not a risk and the instrument response is stable over the course of the analysis or when mixing with acid will create crystallization/precipitation of eluent components and/or analytes.[104]

4.1.3. Single-particle ICP-MS and coupling with AF4. Optimizing spICP-(TOF)MS-based techniques involves evaluating the transport efficiency using appropriate (nano) particle standards; adapting the spray chamber type, the dwell time and the sample dilution for adequate individual particle detection; limiting the background contribution; and evaluating its contribution. Finally, calibrating the ICP-MS signal is required for quantitative estimation of NNP concentration, their elemental content (and size if necessary), and frequency distribution. These experimental and data treatment considerations can be found in the literature[195–197] and are visualized in Fig. 5.

For spICP-TOFMS, the additional benefit of multi-element analysis necessitates careful optimization of the TOF mass analyzer to maintain high resolution and sensitivity. Blanking out certain mass ranges with high concentrations can help prevent detector overloading and potential damage. These steps are crucial for accurately determining particle size distributions, concentrations, and elemental compositions. The spICP-TOFMS instrument can be operated in two modes: standard operation and collisional cooling mode. In standard

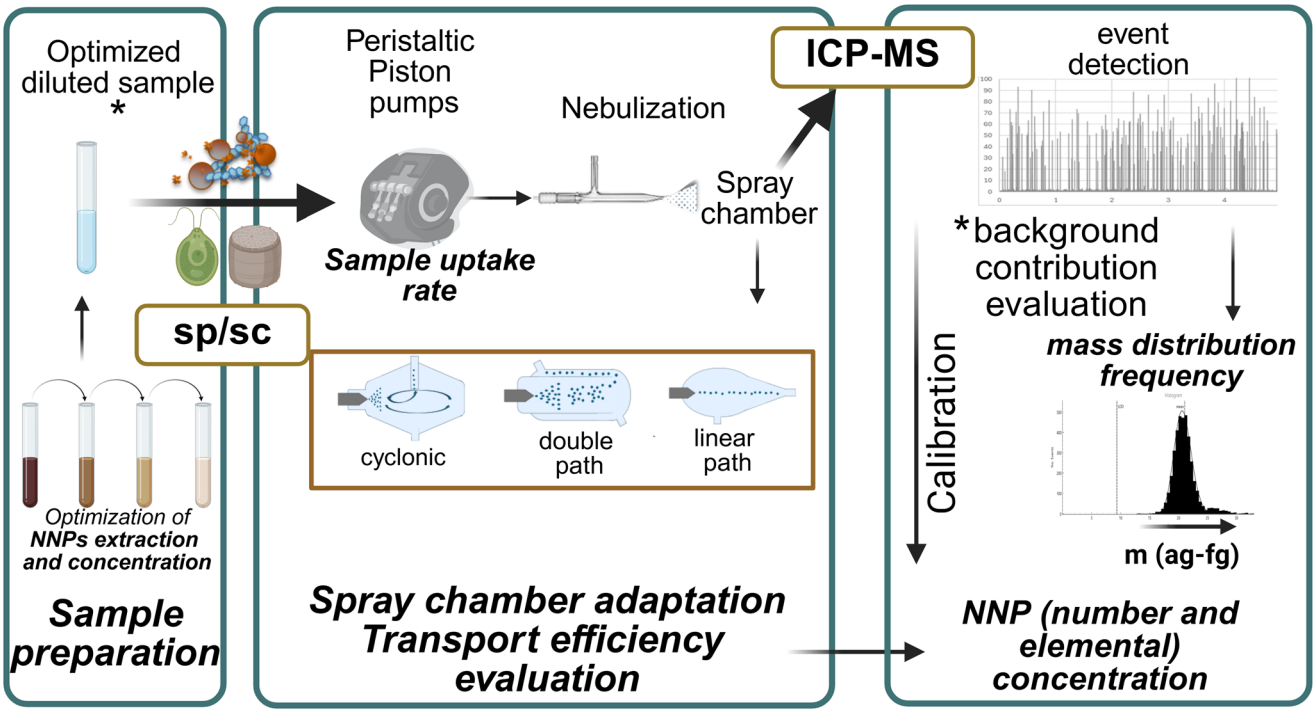


Fig. 5 Important considerations for optimizing the analysis of NPS using spICP-(TOF)MS.

mode, without gas in the collision cell, the system achieves a balance of sensitivity, ion transmission, and mass-resolving power across a mass range of 14–254 *m*/*z*. This mode is typically suitable for routine analyses, allowing simultaneous measurement of elements Li^+ to UO^+. Collisional cooling mode is achieved by pressurizing the collision cell with helium, which enhances high *m*/*z* sensitivities through collisional focusing of the ion beam. This configuration also improves the mass resolving power to values greater than 4000, facilitating high-resolution multi-element analysis. For applications requiring the detection of low *m*/*z* ions, adjustments to the radio frequency waveforms of the collision cell and notch filter enable transmission of ions down to 7 *m*/*z*. However, these low-pass settings reduce the transmission of high-mass ions, limiting the mass range to 7–175 *m*/*z*. This trade-off requires careful tuning to balance sensitivity and mass range coverage depending on the analytical requirements.[198] Acceptable and stable transport efficiency are also required to calculate the mass of NPs using event intensity and frequency, which can be calculated using any of three methods.[199]

While ICP-MS analysis of bulk aqueous samples has largely been optimized as described above, there are significant gaps remaining in the optimization of spICP-MS operation and data processing. One significant limitation is the lack of internal standards (ISTD) in most spICP-MS studies, although this is becoming more common.[200–203] ISTD are essential for quantifying and correcting instrumental drift and matrix effects and can be readily applied to solution analysis with any of the mass analyzers described above. However, the addition of an ISTD in spICP-MS analysis is more complex, with only limited applications in conventional aqueous approaches (*i.e.*, aqueous solutions mixed with ISTD using a mixing tee).[204] Recently, the analysis of monodisperse microdroplets containing aqueous standards has been developed as a promising alternative. In an aqueous standard, a droplet of a known size will contain a precise mass of each element in the standard. This effectively acts as a standardized particle and can be applied to any element.[205] When droplets fall directly into the plasma, their transport efficiency is 100%. In recent applications, a microdroplet generator (MDG) has been used in tandem with a conventional nebulizer for aqueous standards and samples, respectively. In this setup, the sample is nebulized into the plasma while a "burst" of microdroplets is simultaneously introduced from the MDG. This allows the signal of the microdroplets to be corrected for the sample matrix, providing reliable and repeatable internal standardization and facilitating NP quantification in difficult matrices such as seawater.[206]

Sample preparation is another key area requiring optimization for successful spICP-MS analysis. Because the spICP-MS signal includes dissolved ions and particle signals, the dilution factor and analysis time must be optimized such that sufficient particles are analyzed and the background signal from dissolved ions is sufficiently low to quantify small NPs. While dilution is the simplest strategy to mitigate the effects of the dissolved background, techniques such as ion exchange chromatography have also been coupled to spICP-MS for this purpose.[207] Until recently, dissolved ions were considered the only source of spICP-MS background signal; however, it is now recognized that large numbers of

small "non-resolved" particles form a background signal that is often greater than that generated from dissolved analytes in most natural samples. In these cases, a multi-dilution strategy has been proposed, with low dilution factors to detect large particles in significant numbers and high dilution factors to quantify small NPs.[208] While this approach is promising, it requires further investigation for a broader range of aquatic systems and has not been widely adopted, so most studies report only one dilution factor.

Online coupling of AF4 with spICP-MS presents additional challenges, particularly when multiple online detectors are positioned before the spICP-MS. For example, MALS and especially DLS detectors may not be sensitive enough to detect the low particle number concentrations required for spICP-MS.[209,210] To address this, an additional analysis at higher particle concentrations can be conducted to gather missing information. Alternatively, particle fractions can be collected at specific time intervals and analyzed using standalone spICP-MS after proper dilution, employing an offline coupling method.[211,212] The flow can also be split in front of the spICP-MS inlet for online sample dilution. Online coupling is less labor-intensive than offline coupling but requires careful optimization of the injected particle number concentration to minimize or avoid particle coincidences in spICP-MS throughout the entire elution process.[108] Achieving this concentration matching is practically challenging and often necessitates multiple runs at varying dilutions to determine the optimal conditions for all particles.

The evolution of spICP-MS has offered better knowledge of the processes occurring during nebulization and the transfer of particles to the torch, which is also beneficial for AF4-ICP-MS coupling in general. These improvements have been helpful in developing AF4-scICP-MS. For example, classical nebulizers let particles pass, minimizing the risk of clogging. However, the dynamics of aspirated droplets inside the spray chamber can alter the transfer of particles to the torch. In recent developments, AF4-MALS-ICP-TOFMS was successfully used to detect P and Pb in exposed yeast cells.[171] This setup allowed the removal of the ionic background *via* passage through the AF4 membrane and diluted the cells entering the ICP thanks to the cyclonic spray chamber; however, the potential co-occurrence of cells was not thoroughly investigated. The efficiency of cell transfer was 0.1%, minimizing the risk of coincidence, but was far lower than reported for NPs with the typical cyclonic or Scott double-pass spray chamber for NPs (6–16%).[213] Compared to the ESI APEX-IR system or the cyclonic spray chamber tested for potential in AF4 coupling with ICP-Q-MS, only the Meinhard direct injection high efficiency nebulizer (DIHEN) enabled the efficient transfer of particles above 4 μm into the ICP-MS, but the DIHEN wasn't suitable for the introduction of particles fractionated by AF4 due to high flow fluctuations.[214] Other types of total consumption chambers with high transfer efficiencies are used for scICP-(TOF)MS analysis but, again, with significantly lower inflow required (10–20 μL min^{-1}) compared to the outflow of AF4 programs used for the characterization of NNPs and NPS.[215]

4.2. Advantages and limitations

Unlike traditional chromatography, AF4 uses an unobstructed channel where the applied flow is minimally tortuous, resulting in reduced shear forces and a gentle separation process.[216] In traditional and size exclusion chromatography (SEC), strong interactions can occur between the stationary phase and the sample. These interactions may lead to significant shear-induced degradation of larger aggregates, irreversible adsorption of smaller molecules and particles, coelution, or denaturation of the sample.[217] Additionally, AF4 offers enhanced capability with increasing molar mass without being restricted by an exclusion limit as in SEC. It supports a wide range of solvents or buffers, allows fractionation at various temperatures, and can be coupled online with numerous detectors to separate and analyze complex, broadly dispersed multicomponent samples with minimal sample preparation. The ability to quickly and precisely adjust flow rates provides fine control over retention, enabling tailored separation and resolution for each sample. However, this exceptional versatility also poses a significant challenge, as the eluent, flow conditions, and individual parameters typically benefit from optimization for each sample type.

While AF4 offers several advantages over other separation and fractionation methods, it also encounters challenges with respect to material losses through membrane pores, particle–membrane interactions, sample dilution, washing of sample components during focusing, and channel overloading. Based on recent developments, AF4-ICP-MS can theoretically measure size-resolved TEs associated with NPs from macromolecules to nano-assemblages, in addition to characterizing the origin of NPs responsible for TE dispersion, across a wide size range for different environmental systems. Our capacity to measure TE is, however, directly related to both the limits of detection for the ICP-MS being used as well as sample preparation, and also depends on intrinsic characteristics of the analyte such as its natural abundance and ionization efficiency. Additionally, concentrations of the elements and NPs of interest can be at trace or ultra-trace levels. These differences in the loading of NPs in an aquatic system are clearly reflected in the choice of injected volumes and the TE that are monitored in AF4-ICP-MS analysis in the literature.

The pre-concentration or in-channel concentration that is often required prior to analyzing low-concentration samples using FFF can alter speciation, especially when dealing with TEs and components weakly adsorbed to NNPs.[218,219] Pre-concentration usually involves reducing sample volume through ultrafiltration or centrifugation, which increases particle concentration and interactions, potentially causing aggregation or changes in their structure. However, the stability of NPs and associated TE during such concentration

procedures has rarely been investigated. Recent reports indicate that the type of ultrafiltration used for pre-concentration may lead to different artefacts such as aggregation and the generation of new aggregates or NP assemblies, especially under tangential flow.[220] Centrifugal ultrafiltration (CUF) better preserved the size distribution with minimum handling time using increasing concentration factors from 10 to 450 times natural concentrations.[221] Retentates obtained from 10 to 100 times concentrations generally preserved the size distribution of colloids measured by AF4-UV-MALS-ICP-MS despite losses of larger NNPs, but enhanced absorption of U on NNPs was observed. Since this procedure was optimized for one type of soil, its application to other environmental settings remains to be explored. A similar problem can occur during in-channel concentration, where particles are concentrated in a small area, promoting aggregation. During the focusing stage, the sample is constantly washed with the carrier flow, which causes weakly sorbed elements to desorb and hence be lost with the carrier fluid flow. As a result, the measurement primarily reflects the non-labile metals bound to the NPs rather than the total metal content.[175] Slot-flow AF4 offers several advantages to mitigate these challenges; however, it may require careful calibration of the ICP-MS, since the AF4 outflow decreases drastically, and it is challenging to estimate the enrichment factor obtained for ICP-MS signals over the size continuum.[104,189,190] An alternative was proposed by adding 1 ppb of the internal standard directly in the carrier solution of AF4, allowing quantitative analysis of the composition of NNPs in the range of 1 to 250 nm.[181] However, a strategy for quantitatively determining TEs that are bound/adsorbed on the surface of NNPs remains to be validated. The dilution of samples that is required for spICP-(TOF)MS may similarly disturb the natural distribution and speciation of trace elements; indeed, concentration-associated changes are a ubiquitous challenge for analytical methods in environmental nanobiogeochemistry.

Finally, fractograms are better interpreted in terms of size-based population or colloid type, each of which can contain several types of species, often with overlapping peaks which require the use of deconvolution to gain in size-selectivity.[100,191] Using this approach, the selectivity of AF4-ICP-MS can elucidate the nature of elements associated with NNPs (*i.e.*, organic *vs.* inorganic in addition to size separation), which changes along the size continuum of fractionation. On the other hand, spICP-MS or spICP-TOFMS measures the inorganic composition of individual NPs when the mass of elements is sufficient to be detected above the background.

Analysis using spICP-TOFMS is a powerful approach for characterizing complex mixtures of nanomaterials without prior knowledge of their composition.[222] This untargeted method allows for the detection of the unique elemental fingerprints of various NNPs. The advantages and limitations of spICP-TOFMS were recently reviewed by several groups.[223,224] A major advantage of spICP-TOFMS is its capability to concurrently detect both particulate and dissolved signals at concentrations relevant to environmental conditions. The method's multiplexed detection capabilities enable high-throughput analysis and quantitative element-ratio measurements at the single-particle level, making it highly versatile for fields such as nanotoxicology, materials science, environmental science, geochemistry, forensics, and cell biology. While its sensitivity for single m/z detection is lower than that of state-of-the-art ICP-QMS and ICP-SFMS systems, spICP-TOFMS compensates with multi-element detection, providing superior selectivity and the ability to fingerprint diverse NP types. Despite these advantages, challenges persist, including distinguishing small particles and small masses of elements from the background signal with associated limits for detecting small particles and minor elements, managing and interpreting the rich datasets, analysis being limited to the inorganic fraction of NNPs, distinguishing true multi-metal NNP events from coincidental detections, and achieving the sensitivity of other ICP-MS systems. Advanced data analysis approaches, including multi-dimensional clustering and machine learning, are expected to address many of these issues, driving spICP-TOFMS from analytical development to broader applications and making it an indispensable tool in the fast-evolving field of environmental nanogeochemistry.[107,112,225,226]

Data availability

The data that support the figures and tables in this manuscript exist as part of numerous research programs undertaken by the co-authors and/or their supervising entities, each with distinct licensing policies. The data are therefore not available in a single location but may be accessed by contacting the corresponding authors.

Conflicts of interest

There are no conflicts of interest to declare.

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