Asymmetrical flow-field-flow fractionation coupled with inductively coupled plasma mass spectrometry for the analysis of gold nanoparticles in the presence of natural nanoparticles

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ABSTRACT

Flow-Field-Flow Fractionation (Flow-FFF), coupled with online detection systems, is one of the most promising tools available for the analysis and characterization of engineered nanoparticles (ENPs) in complex matrices. In order to demonstrate the applicability of Flow-FFF for the detection, quantification, and characterization of engineered gold nanoparticles (AuNPs), model dispersions were prepared containing AuNPs with diameters of 30 or 100 nm, natural nanoparticles (NNPs) extracted from a soil sample, and different concentrations of natural organic matter (NOM), which were then used to investigate interactions between the AuNPs and the NNPs. It could be shown that light scattering detection can be used to evaluate the fractionation performance of the pure NNPs, but not the fractionation performance of the mixed samples that also contained AuNPs because of specific interactions between the AuNPs and the laser light. A combination of detectors (i.e. light absorbance and inductively coupled plasma mass spectrometry (ICP-MS)) was found to be useful for differentiating between heteroaggregation and homoaggregation of the nanoparticles (NPs). The addition of NOM to samples containing 30 nm AuNPs stabilized the AuNPs without affecting the NP size distribution. However, fractograms for samples with no added NOM showed a change in the size distribution, suggesting interactions between the AuNPs and NNPs. This interpretation was supported by unchanged light absorption wavelengths for the AuNPs. In contrast, results for samples containing 100 nm AuNPs were inconclusive with respect to recovery and size distributions because of problems with the separation system that probably related to the size and high density of these nanoparticles, highlighting the need for extensive method optimization strategies, even for nanoparticles of the same material but different sizes.

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1. Introduction

Engineered nanoparticles (ENPs) are being increasingly used in consumer products. Metal and metal oxide nanoparticles, consisting for example of Ag, Au, ZnO, and TiO_2 and carbon-based nanomaterials such as fullerenes and carbon nanotubes, are currently the most commonly used materials [1]. Gold nanoparticles (AuNPs) are mainly used for biomedical applications [2–4] or in sensing devices [e.g. 5–8] and are available as analytical standard materials [9]. ENPs can be released from products in which they are used into the environment [10–13]. ENPs released into aquatic and terrestrial environments can aggregate, undergo transformations [14–17], or interact with a complex background of natural nanoparticles (NNPs) that may include clay or oxide particles and natural organic matter (NOM) [15,18–21]. The NNPs are present in much higher (mass) concentrations than the ENPs and have the potential to dominate the fate and behavior of the ENPs [16]. It has for example been shown that natural organic matter (NOM) can replace citrate coatings on AuNPs, enhancing their dispersion stability [22,23], and recent systematic investigations into the fate and behavior of ENPs in aquatic systems have shown that the behavior of such particles is mainly influenced by their surface functionalization [23,24].

However, methods for the detection, quantification, and characterization of ENPs within such complex environmental matrices are either generally absent or still under development [25,26]. Dispersed nanoparticles (NP) systems are metastable systems and low

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perturbing methods are therefore required to address these tasks. The use of Flow-Field-Flow Fractionation (Flow-FFF) coupled to a detection system such as inductively coupled plasma mass spectrometry (ICP-MS) is a promising technique for the detection and quantification of ENPs in complex matrices because of its particle size related separation power, its versatility, and its elemental specificity [27,28]. In brief, Flow-FFF is a technique for the continuous separation of particles and macromolecules with diameters between 1 and 1000 nm, which is based on hydrodynamic principles and avoids the use of a stationary phase. Details concerning its principles of operation and applications have been published elsewhere [27,29–32].

Flow-FFF has been widely applied to analyze NNPs, e.g. when investigating interactions between trace elements and NNPs within complex environmental matrices [30,32–34]. The use of Flow-FFF for ENPs such as AuNPs or silver NPs (AgNPs) has, to date, mainly focused on aquatic systems and stability studies [35–38]. Few studies have focused on the analysis of metallic ENPs extracted from biological material, foodstuffs, or organic tissue [26,39–41]. Very few reports have been published on the use of Flow-FFF methods for analyzing ENPs in complex environmental matrices such as soil and sediment, or for investigating interactions between metallic ENPs and NNPs [42].

For Flow-FFF analysis of ENPs in complex samples (such as soils) the NPs first need to be extracted from the matrix and transferred into a stable aqueous dispersion. The resulting dispersion will contain not only ENPs but also NNPs such as clay particles, oxides, and varying amounts of NOM, which will affect the recovery and aggregation state of both ENPs and NNPs [23,43–45].

The objective of this study is to demonstrate the applicability of Flow-FFF multidetection for the detection, quantification, and characterization of metallic ENPs in the presence of NNPs. For this purpose we used mixtures of different sized AuNPs, with sizes at the lower and upper end of the nano-size-range (1–100 nm), with NNPs extracted from a soil sample [34,46] as model dispersions. The effect of different NOM concentrations on the stability and interactions of the NPs was investigated by analyzing dispersions with added NOM, and dispersions without additional any NOM.

2. Materials and methods

2.1. Chemicals

The Milli-Q water used in this study was prepared using a Millipore Advantage A10 system (Millipore, Billerica, USA) equipped with a Bio-Pak^{TM} Ultrafilter (5000 g mol^{-1} molecular weight cut-off) for final clean-up. Sodium pyrophosphate (analytical grade) was purchased from Merck (Darmstadt, Germany), as were the HNO$_3$ (68% suprapure) and HCl (30% suprapure) used for aqua regia digestion.

Aqueous suspensions of citrate-stabilized AuNPs with nominal diameters of 30 nm (30 nm AuNPs) and 100 nm (100 nm AuNPs) were purchased from BBI International (Cardiff, UK). The Au concentrations in the 30 nm AuNP and 100 nm AuNP suspensions were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis (Perkin Elmer Optima 5300 DV, PerkinElmer, Beaconsfield, UK) following digestion with concentrated aqua regia solution at 80 °C, and were found to be 63 mg L^{-1}

and 50 mg L^{-1}, respectively. Suwannee River natural organic matter (SRNOM) was purchased from the International Humic Substance Society (St. Paul, MN, USA). Nanosphere^{TM} latex beads of 40, 80, 200 and 400 nm diameter were purchased from Duke Scientific (Palo Alto, CA, USA) for calibration of the channel.

A sample of NNPs from a colloidal soil extract, with a particle mass concentration of 200 mg L^{-1}, was used as a model system for a complex NNP matrix [46]. The wet extraction procedure used to prepare the NNP extract has been previously described in [18]. The extract contains particles with sizes ranging between 10 and 400 nm [46] and is routinely used as an in-house reference sample to validate and compare the performance of Flow-FFF analytical methods [34,47].

2.2. Samples

Two series of aqueous samples containing AuNPs (at a calculated particle mass concentration of 2 mg L^{-1}) and NNPs (at a calculated particle mass concentration of 143 mg L^{-1}) were investigated in this study. The samples were prepared by mixing the AuNP stock suspensions with the NNP suspension and diluting the mixture with Milli-Q water. The samples were then stored cool (4 °C) and dark place for two months prior to taking any measurements, in order to ensure complete interaction. Samples were prepared both with and without the addition of SRNOM (final calculated concentration 28 mg L^{-1}) in order to investigate the influence of NOM on the stability of the AuNP-NNP system. Total Au concentrations were determined following aqua regia digestion; the nominal Au and NNP concentrations of the samples are shown in Table 1.

2.3. Asymmetric Flow-FFF-UV/VIS-MALLS-ICP-MS

A Wyatt Eclipse 3+ Flow-FFF system (Wyatt Technology Europe, Dernbach, Germany) was used for the fractionation of the samples. The channel had a length of 29.6 cm and was equipped with a 10 kDa MWCO regenerated cellulose membrane (NADIR, Wiesbaden, Germany). The carrier solution was delivered by an Agilent 1200 series quaternary HPLC pump equipped with a micro vacuum degasser. Carrier solutions were filtered with Anodisc 0.02 μm membrane filters (Whatman, Maidstone, UK) prior to use. The outflow was analyzed using a UV/Vis diode array detector (Agilent Technologies 1200 series DAD; absorption wavelengths selected: 260, 280, 540 nm), multi angle laser light scattering MALLS (DAWN HELEOS II, Wyatt Technologies, Dernbach, Germany), and ICP-MS (Agilent Technologies 7700x, Waldbronn, Germany). The channel recovery was determined by injecting samples without applying any cross flow. The details of the Flow-FFF and ICP-MS run conditions and parameters are summarized in Table 2. The ICP-MS was calibrated by manual injection of metal standard solutions via a Rheodyne 9725i switch valve (Rheodyne, CA, USA) between the channel outlet and the ICP-MS interface. In addition to Au, Fe and Al were also chosen as representative elements for the NNP sample, which mainly contains oxides (e.g. iron oxides) or clay minerals.

2.4. Data treatment

The Flow-FFF separation of the samples was evaluated using MALLS analysis and by calculating radius of gyration values. The

Table 1
Nominal concentrations of AuNPs and NOM, particle mass concentrations of NNPs in mixed samples.

Sample	AuNP diameter (nm)	[Au] nominal (mg L^{-1})	[NNP] (mg L^{-1})	[NOM] (mg L^{-1})
Au30 + NNP	30	2	143	
Au30 + NNP + NOM	30	2	143	28
Au100 + NNP	100	2	143	
Au100 + NNP + NOM	100	2	143	28