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Separation and quantification of silver nanoparticles and silver ions using reversed phase high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry in combination with isotope dilution analysis

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ABSTRACT

A reversed phase high performance liquid chromatography coupled to an inductively coupled plasma mass spectrometer (HPLC–ICP-MS) approach in combination with isotope dilution analysis (IDA) for the separation and parallel quantification of nanostructured and ionic silver (Ag) is presented. The main focus of this work was the determination of the ionic Ag concentration. For a sufficient stabilization of the ions without dissolving the nanoparticles (NPs), the eluent had to be initially optimized. The determined Ag ion concentration was in a good agreement with results obtained using ultrafiltration. Further, the mechanism of the NP separation in the HPLC column was investigated. Typical size exclusion effects were found by comparing results from columns with different pore sizes. Since the recovery rates decreased with increasing Ag NP size and large Ag NPs did not elute from the column, additional interactions of the particles with the stationary phase were assumed. Our results reveal that the presented method is not only applicable to Ag NPs, but also to gold and polystyrene NPs. Finally, IDA-HPLC-ICP-MS experiments in *single particle* mode were performed to determine the particle cut-off size. The comparison with conventional spICP-MS experiments resulted in a similar diameter and particle size distribution.

1. Introduction

Silver nanoparticles (Ag NPs) are widely used in consumer products, such as coatings of refrigerators, cosmetics and clothing, to avoid bacterial growth. The growing use of Ag NPs in consumer products leads to an increasing exposure to humans and nature [1]. Studies show that NPs might be released from these products [2]. As their environmental fate is not fully understood up to now, a great research interest has emerged. The effect of environmental conditions, such as natural organic substances, pH value or salts, was shown to be species-dependent, therefore affecting NP stability, aggregation state and dissolution rate [2–4].

Multiple studies on the toxicity of Ag NPs reveal that they are an effective biocide against bacteria [5–8], fungi [3,6,8], viruses [8,9], and are also toxic to algae [3,10]. The mechanism of the toxicity is supposed to be based on the release of Ag ions [5,7,10,11]. In terms of bacteria, Ag ions can inhibit the P-, S-, and N-cycle of nitrated bacteria. They can also block DNA transcription, respiration, and disturb the adenosine triphosphate synthesis. Some studies reported the ability of nanosilver to enhance the formation of reactive oxygen species [6,7,11] and harm the cell membrane [5,12,13]. It is assumed that the toxicity is species-dependent in terms of NP size, surface coating, aggregation behavior, and solubility but independent of the total Ag mass [14–19]. A thorough characterization of the used Ag NPs and especially its ionic concentration is therefore crucial to evaluate the potential effects on humans and the environment [20].

Fabricius et al. illustrated the great variety of concentration values which can be obtained for the Ag ions of the same Ag NP sample using cloud point extraction (CPE), centrifugation, dialysis,

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ultrafiltration, tangential flow filtration (TFF), and an ion-selective electrode. These values ranged from approximately 7% (TFF) to 36% (dialysis for 12 days) [21]. Silver ions can be lost on the filters used during ultrafiltration or remain in the supernatant during CPE. The removal of Ag^+ from the Ag NPs in osmosis can cause their dissolution. Eq. (1) shows the dissolution mechanism in water in the presence of oxygen.

$$2Ag_{(s)} + \frac{1}{2}O_{2(aq)} + 2H^+_{(aq)} \rightleftharpoons 2Ag^+_{(aq)} + H_2O_{(l)} \quad (1)\,[11]$$

This reaction is likely to form a hydrogen peroxide intermediate. Liu et al. showed for citrate stabilized Ag NP with a diameter of 4.8 nm and a concentration of 2 mg L^{-1} dissolution of approximately 1.6% in a quarter day in the dark in air-saturated conditions [11]. Therefore, an under- or overestimation of the Ag ions can be easily obtained [21,22]. Additionally, these techniques are laborious and time-consuming. Consequently, a fast technique, which is able to separate the ionic Ag from the Ag NPs and quantify both species in one step is needed. Hyphenated techniques, combining separation with suitable detectors are able to overcome these challenges.

Asymmetrical flow field-flow fractionation (AF4) in combination with ICP-MS is often used to determine the particle size and the concentration of Ag NPs [23,24]. However, ionic Ag can pass the membrane due to its small size and can therefore not be detected using this technique [25]. Hydrodynamic chromatography provides a poor resolution but is able to separate Ag NPs from Ag ions [26]. It is often combined with ICP-MS in *single particle* (sp) mode in order to determine the geometric diameter of the metal core [27,28]. Due to the low concentration, a quantification of the ions is challenging using this approach.

Capillary electrophoresis (CE) in combination with ICP-MS has recently been applied to separate Ag NPs from ionic Ag. Franze et al. showed a baseline separation of ionic Ag, 10 nm and 30 nm Ag NPs. As this technique is sensitive to the particle surface charge, particles of the same size but a different surface coating have a different interaction with the surfactant and therefore influence the migration. This can be a challenge for the analysis of complex and/or unknown NP samples [29]. Hanley et al. uses cation exchange chromatography to separate Ag NPs from ionic Ag [30]. However, all particles smaller than 100 nm were not separated. Thus, a size determination of the Ag NPs was not possible. Zhou et al. proposed a HPLC–ICP-MS method using an amino-modified stationary phase. This method is able to separate ionic Ag and Ag NP up to 100 nm diameter [31], but the chromatographic resolution was quite poor. Additionally, a method validation for the quantification of the concentration of the ionic Ag is lacking.

Helfrich et al. presented a reversed phase HPLC–ICP-MS approach with the ability to determine particle diameters of Au NPs using an external calibration of particles with a known size [32]. By comparing the elution of a NP mixture using columns with a 300 Å and a 1000 Å pore size, the separation mechanism was found to correspond to size exclusion chromatography (SEC) [33]. Soto-Alvaredo et al. applied a similar method for the analysis of Ag NPs in sport socks [34]. However, a method for quantification is still lacking. Therefore, information about the recovery rates and the amount of ionic Ag is not available.

In this work, we applied a combination of isotope dilution analysis (IDA) with a modified HPLC method reported by Soto-Alvaredo et al. [34]. This calibration approach acts both as an internal and calibration standard and can therefore compensate possible matrix effects. The objective of this work was to develop a method for a simultaneous quantification of Ag NPs and ionic Ag. The separation mechanism will be investigated by comparing columns with different pore sizes. The results will be validated in additional experiments using Au and polystyrene (PS) NPs.

2. Material and methods

2.1. Chemicals

Elemental Ag and In standards (CertiPUR grade), isotopically enriched spike solution Ag-109 (CertiPUR grade, 9.53 mg L^{-1} Ag, 98.3% ^{109}Ag) and ammonium acetate (p. A. grade) were purchased from Merck (Darmstadt, Germany). DL-Penicillamine (p. A., 99+ %, gold grade) was obtained from Aldrich (Aldrich Chemie, Steinheim, Germany) and sodium dodecyl sulfate (SDS, Biochemica grade) from AppliChem GmbH (Darmstadt, Germany).

Citrate stabilized Ag NPs with nominal diameters of 20 nm, 30 nm and 40 nm were purchased from Nanocomposix Europe (Prague, Czech Republic), citrate stabilized Ag NPs with a nominal diameter of 10 nm from Sigma Aldrich (Munich, Germany) and Au NP reference materials 8011, 8012 and 8013 with nominal diameters of 10 nm, 30 nm and 60 nm from NIST (Gaithersburg, MD, USA). Plain PS NPs with mean nominal diameters of 20 nm and 50 nm were obtained from Postnova Analytics (Landsberg am Lech, Germany). Additionally, the in-house certified reference material Ag NPs BAM N-001 (BAM, Berlin, Germany) was used. For simplicity reasons these materials are further denoted as NCx, Sx, NISTx and PSx, with x corresponding to the nominal particle diameter.

Ultrapure Water (18.2 MΩ cm, Millipore Element system equipped with a Quantum ICP Polishing Cartridge, EMD Millipore, Billerica, MA, USA) was used for the preparation of the HPLC eluent and the sample solutions.

Argon gas (99.999%, 5.0) used for the operation of the ICP-MS, was purchased from Linde AG (Pullach, Germany).

2.2. HPLC–ICP-MS hyphenation

A Knauer Smartline HPLC (Knauer GmbH, Berlin, Germany) was connected to an iCAP Q ICP-MS instrument (Thermo Fisher Scientific, Bremen, Germany). Two reversed-phase Nucleosil C18 columns with a particle size of 7 μm, a pore size of 1000 Å and 4000 Å, a length of 250 mm and an inner diameter of 4.6 mm were chosen (Macherey-Nagel GmbH & Co. KG, Düren, Germany). An isotopically enriched ^{109}Ag standard with a concentration of 1.91 μg L^{-1} was added post-column for quantification using an external pump (LabCraft, Saint-Andre-des-Eaux, France) with a flow rate of 94 μL min^{-1}. A mixture of 10 mmol L^{-1} SDS, 10 mmol L^{-1} ammonium acetate, penicillamine (PA) and a pH 6.7 with a flow of 0.5 mL min^{-1} was used as eluent. During its optimization, PA concentrations of 100 μmol L^{-1}, 250 μmol L^{-1}, 500 μmol L^{-1} and 1000 μmol L^{-1} were tested. The eluent was filtered (0.2 μm polyethersulfone syringe filter, Rotilabo, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and degassed in an ultrasonic bath for 30 min prior to use. The HPLC column was operated at room temperature.

For the detection of the PS NPs, a SPD–20 M diode array detector (DAD, 254 nm) from Shimadzu (Kyoto, Japan) was used instead of the ICP-MS.

Table 1 summarizes the experimental ICP-MS conditions.

2.3. HPLC-spICP-MS

For the HPLC–ICP-MS experiments in sp-mode, a sample concentration of approximately 0.5 μg L^{-1} and the 1000 Å column were chosen. Six experiments were performed using the NC40 NPs. Since these particles eluted at retention times between 207 s and 300 s in a regular HPLC-ICP-MS experiment, all events between 200 s and 310 s were evaluated. The determination of the particle size was performed as previously reported [35].