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Separation of silver ions and starch modified silver nanoparticles using high performance liquid chromatography with ultraviolet and inductively coupled mass spectrometric detection ☆

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ABSTRACT

The production of commercially available products marketed to contain silver nanoparticles is rapidly increasing. Species-specific toxicity is a phenomenon associated with many elements, including silver, making it imperative to develop a method to identify and quantify the various forms of silver (namely, silver ions vs. silver nanoparticles) possibly present in these products. In this study a method was developed using high performance liquid chromatography (HPLC) with ultraviolet (UV–VIS) and inductively coupled mass spectrometric (ICP-MS) detection to separate starch stabilized silver nanoparticles (AgNPs) and silver ions (Ag^+) by cation exchange chromatography with 0.5 M nitric acid mobile phase. The silver nanoparticles and ions were baseline resolved with an ICP-MS response linear over four orders of magnitude, 0.04 mg kg^{-1} detection limit, and 90% chromatographic recovery for silver solutions containing ions and starch stabilized silver nanoparticles smaller than 100 nm.

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1. Introduction

Silver nanoparticles are commonly incorporated into consumer products [1] because of the broad-spectrum antimicrobial properties of silver [2]. Recent studies indicate that AgNPs degrade and silver is released from textiles and consumer products containing AgNPs [3–6]. From an ecological and human health prospective, the cellular mobility and toxicity of AgNPs differ from their bulk silver counterpart [7]. It is generally accepted that the toxicity of silver is associated with the silver ion (Ag^+) form [8]. However, AgNPs have shown an increased toxicity in prokaryotes compared to Ag^+ because of the increased mobility of AgNPs across the cellular membrane resulting in the increased Ag^+ release [9,10]. Since the biological activity of silver is dependent on its chemical form, the ability to detect Ag^+ and AgNPs at relevant biological and environmental concentrations is required to accurately assess the benefits and risks associated with incorporating AgNPs into consumer products [11,12].

A variety of methods are used to characterize nanoparticles in commercial products. Aside from visualizing the nanoparticles using transmission electron or scanning electron microscopy (TEM, SEM) [13,14], particles can be detected using dynamic light scattering [15] (DLS) or UV–VIS spectroscopy [16,17]. The particles undergo Brownian motion, which leads to Raleigh scattering for particles smaller than the incident wavelength of light. Using DLS the scattering can be measured and correlated to a diffusion coefficient ultimately leading to particle size determination [18]. Since AgNPs have unique optical properties, a great deal of information about the AgNP state and size in solution can be obtained through monitoring their spectral properties. There is a positive correlation between AgNP size and corresponding UV–VIS wavelength. As AgNPs increase in size, the wavelength of light they scatter also increases. This response can be attributed to scattering solely from the silver core opposed to any functional group on the AgNPs [16]. These methods offer particle specific detection but cannot detect the bulk non-nano counterpart. A method capable of detecting both the AgNPs and Ag^+ would be ideal.

Emerging techniques have demonstrated the capability of separating nanoparticles from other nanoparticles and bulk material including ion forms [19]. These methods include AgNP size separation or separating AgNPs from bulk silver using field-flow fractionation (FFF) [20], single particle detection (sP) [21], cloud-point extraction (CPE) [22], or reverse phase and size exclusion chromatography [23]. The majority of these separations have been coupled with ICP-MS [21–25]. The emergence of size exclusion and reverse phase chromatography to separate nano and non-nano forms of silver offers the advantages of higher throughput, matrix tolerance, and the use of multiple nano-specific detectors (such as UV–VIS or DLS) added in tandem with ICP-MS detection. Traditionally, cation exchange chromatography (CEX) with an acid mobile phase has been used to separate Ag^+ from other

☆ This paper is dedicated to Nicoló Omenetto, on the occasion of his 75th birthday, in recognition of his outstanding contributions to the field of laser spectrochemistry and as an editor of Spectrochimica Acta Part B.

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elements [26]. Gautier et al. developed a method to separate silver from cadmium and palladium using a carboxylate stationary phase [26]. The CEX method provided excellent column recoveries to quantify Ag^+ but was not used for the separation of Ag^+ plus AgNP.

A variety of commercially marketed products labeled to contain AgNPs are available. Although silver is not recognized by the United States Food and Drug Administration as a safe or effective over the counter drug to treat ailments, colloidal silver dietary supplements are commercially available [27]. Colloidal silver is not thought to be harmful to humans but chronic consumption of silver and colloidal silver can result to Agyria where the skin becomes permanently discolored [28]. Often colloidal silver dietary supplements are marketed to contain AgNPs and labeled to contain minimal ingredients including distilled water, nanosilver, and an agent used to stabilize the nanoparticle. Through ingestion, these products claim to "aid in immune support" among other claims [28]. The health effects related to using colloidal silver were not tested during this study; rather the ability to detect nanoparticles in a locally purchased colloidal silver dietary supplement was demonstrated.

Starch modified AgNPs were chosen for this study because potato starch is generally recognized as a safe (GRAS) commodity by the FDA. Using a GRAS stabilization agent sets the ground-work for a method that can be used to characterizing consumer silver nano-products, and can also be used for toxicity studies to assess the ecological and health implications AgNP products may impose. Additionally, using a green method for synthesis eliminates the need for additional reducing agents and ultimately limits the chemicals present. In this study, colloidal silver dietary supplements were used to demonstrate how CEX in combination with tandem UV–VIS and ICP–MS detection may be implemented to differentially detect starch modified silver nanoparticles and free silver ions (Ag^+) in a commercially available nano-product.

2. Experimental

2.1. Reagents

All reagents were prepared in 18 MΩ ultrapure deionized water (DIW, Milli-Q water purification system, Millipore, Bedford, MA,) Ethanolamine was purchased from Sigma Aldrich (St. Louis, MO, USA). Starch stabilized AgNPs were purchased from Strem Chemicals (Newburyport, MA, USA). High-purity nitric acid, high-purity hydrochloric acid, potato starch and silver nitrate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). A silver secondary check standard (as $AgNO_3$) was purchased from SPEX CertiPrep (Metuchen, NJ, USA). NIST SRM 1643e water was used as a calibration check standard for total silver determination. A silver colloid dietary supplement functionalized with casein was locally purchased (Cincinnati, OH, USA).

2.2. Instrumentation

Dynamic light scattering (DLS) was performed with a Microtrac dynamic light scattering system (York, PA, USA) for nanoparticle size approximation. Microwave digestions were performed with the Mars Xpress (CEM, Matheson NJ, USA) closed vessel system for total silver determination. The separation/characterization of silver forms was performed using an Agilent 1100 or 1290 HPLC system (Santa Clara, CA, USA) each equipped with a binary pump, a micro-membrane degasser, a column compartment with a thermostat and an internal switching six port valve, and a diode array UV–VIS detector for AgNP surface plasma resonance determination. Chromatography was performed with a Dionex IonPac CG12a (3 × 30 mm, 5 μm particle) and CS12 (4 × 250 mm, 8 μm particle) chromatographic columns (Sunnyvale, CA, USA). The CG12a is the corresponding guard column to the CS12, both contain a carboxylic acid stationary phase. Silver species were detected by coupling the HPLC to an Agilent 7700ce ICP-MS or Agilent 8800c ICP-QQQ by connecting the end of the HPLC column to the nebulizer using polyether ether ketone (PEEK) tubing (0.12 mm diameter, Upchurch Scientific, WA, USA). Silver isotopes, ^{107}Ag and ^{109}Ag, were monitored using the ICP-MS without gas in the octopole reaction system. Both Agilent ICP-MS systems were equipped with a CETAC ASX500 autosampler for total metal determination. The Agilent Technologies ICP-MS software (MassHunter G7201A version A.01.02 or G7201B version B.01.02) was used for both total metal/chromatographic data analysis. PEEK tubing was used when possible to reduce memory effects from silver and corrosion from 0.5 M nitric acid mobile phase. The Agilent 1100/1290 HPLCs were equipped with 0.12 mm PEEK lines in combination with a PEEK carboxylic acid functionalized chromatographic column. High-density polyethylene (HDPE) sample preparation and autosampler vials were used to avoid silver adsorption to the vials, which may occur with glass.

2.3. Synthesis of starch stabilized AgNPs

In addition to the starch stabilized AgNPs purchased (approximately 100 nm), a second source of starch–AgNPs was synthesized to validate the efficacy of this procedure and suitability for smaller AgNPs (approximately 10 nm, size determination will be discussed later) since they were not commercially available. Using heat from an autoclave, potato starch was used to both reduce Ag^+ (from aqueous silver nitrate) and stabilize the resulting AgNPs. This method was adapted from Vigneshwaran et al. [29]. Approximately 5 mL of an aqueous solution containing 10 mg mL^{-1} of potato starch and 1 mM $AgNO_3$ was mixed in a Teflon lined 35 mL Pyrex vessels. The vessels were capped and heated in an autoclave for 5 min at 120 °C and 15 psi. The commercially purchased AgNP and the synthesized AgNP were not further purified.

2.4. Total silver analysis

Total silver concentrations were established by the combination of microwave digestion and ICP-MS analysis. Colloidal silver solutions were sonicated as a precautionary measure to reduce agglomerates and aid in AgNP dispersion, then approximately 0.5 g of solution was weighed directly into 100 mL Mars Xpress Teflon digestion vessels. Samples were prepared in quadruplicate where the fourth replicate was fortified with 100 mg kg^{-1} silver. Method blanks and fortified blanks were included during digestion. Approximately 5 mL concentrated nitric acid and 2.5 mL concentrated hydrochloric acid were added to each vessel then the loosely capped vessels containing a sample were allowed to predigest overnight in a laminar flow hood. Resulting solutions were microwave digested in a closed system using 1600 W and ramping up to 200 °C over 10 min then holding at 200 °C for 15 min. After the digested samples cooled, each solution was transferred to 50 mL metal-free HDPE vials and diluted to 25 g with DIW. A second dilution was necessary, diluting 100 mg of dilution 1 to a final mass of 10 g with an aqueous solution containing 300 mM ultra-pure HNO_3 and 5 mM ultrapure HCl.

The second dilution of each sample was analyzed by ICP-MS using an online internal standard solution of 200 μg kg^{-1} of ^{103}Rh in 300 mM ultra-pure HNO_3 and 5 mM ultrapure HCl introduced by a mixing tee, which provided approximately 20× internal standard dilution. A six-point external calibration curve was used to quantify silver from 0.5 μg kg^{-1} to 100 μg kg^{-1}. NIST water standard reference material 1643e was analyzed concurrently with samples along with a secondary check silver standard. ICP-MS parameters can be found in Table 1.

2.5. Silver nanoparticle characterization

The colloidal samples were determined to contain nanoparticles using DLS and UV–VIS diode array analysis. For size approximation, colloidal samples were diluted 10× and then were analyzed using DLS. Complementary to DLS, the surface plasma resonance of these colloidal samples was determined using UV–VIS diode array analysis. Samples