Three-dimensional printed knotted reactors enabling highly sensitive differentiation of silver nanoparticles and ions in aqueous environmental samples

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HIGHLIGHTS

- 3D printed knotted reactors are utilized to differentiate AgNPs and Ag$^+$ ions.
- Xanthan/phosphate-buffered saline is used for stabilizing the two silver species.
- Extraction efficiency up to 54.5% is available for retaining Ag$^+$ ion species.
- The proposed method is more reliable than does a conventional filtration method.

GRAPHICAL ABSTRACT
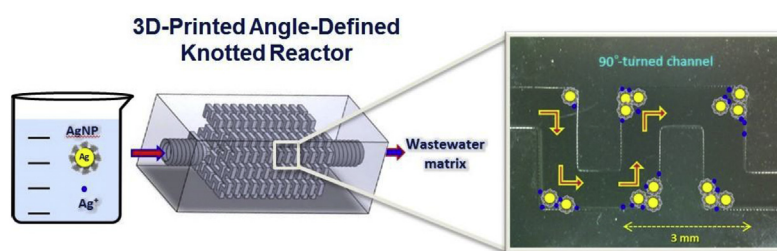
ABSTRACT

Whether silver nanoparticles (AgNPs) persist or release silver ions (Ag$^+$) when discharged into a natural environment has remained an unresolved issue. In this study, we employed a low-cost stereolithographic three-dimensional printing (3DP) technology to fabricate the angle-defined knotted reactors (KRs) to construct a simple differentiation scheme for quantitative assessment of Ag$^+$ ions and AgNPs in municipal wastewater samples. We chose xanthan/phosphate-buffered saline as a dispersion medium for *in situ* stabilization of the two silver species, while also facilitating their extraction from complicated wastewater matrices. After method optimization, we measured extraction efficiencies of 54.5 and 32.3% for retaining Ag$^+$ ions and AgNPs, respectively, in the printed KR (768-turn), with detection limits (DLs) of 0.86 and 0.52 ng L^{-1} when determining Ag$^+$ ions and AgNPs, respectively (sample run at pH 11 without a rinse solution), and 0.86 ng L^{-1} when determining Ag$^+$ ions alone (sample run at pH 12 with a 1.5-mL rinse solution). The proposed scheme is tolerant of the wastewater matrix and provides more reliable differentiation between Ag$^+$/AgNPs than does a conventional filtration method. The concept and applicability of adopting 3DP technology to renew traditional KR devices were evidently proven by means of these significantly improved analytical performance. Our analytical data suggested that the concentrations of Ag$^+$ ions and AgNPs in the tested industrial wastewater sample were both higher than those in domestic wastewater, implying that industrial activity might be a main source of environmental silver species, rather than domestic discharge from AgNP-containing products.

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1. Introduction

The discharge of silver nanoparticles (AgNPs) into our living environment is an inevitability because many AgNP-containing products are being produced and consumed, thanks to their great antibacterial abilities [1—6]. For example, mechanical washing of silver- or AgNP-treated textiles releases up to 30% of the AgNPs or their oxidative species, silver ions (Ag^+), into the washing liquid [7—9]. Although there is no direct evidence linking the use of AgNPs in consumer products to adverse effects on human health, the distribution, accumulation, persistence, and transformation of these discharged AgNP-related species in the natural environment are known to have toxic effects on both terrestrial and aquatic species [9—13]. Moreover, the bioaccumulation and bio-magnification effects of these transformed silver species to bio-logical species at the top of the food chain might seriously influence the ecotoxicological impacts and safety considerations of the widespread use of AgNPs [13—15].

The proposed toxic mechanisms of AgNPs in biological systems include (i) oxidative stress generated through formation of reactive oxygen species (e.g., O_2^-) at their surface and (ii) disturbing the functions of sulfur- and phosphorus-containing biomolecules (e.g., DNA, proteins) through interactions with intact AgNPs or their released Ag^+ ions [11,16]. Although both mechanisms eventually lead to cell dysfunction and death, the toxic responses to biotas are distinct for the two different silver species. For prokaryotes, fresh-water and marine invertebrates, and fishes, ionic Ag^+ is considered to be the most toxic form; the nanoscale-specific properties of AgNPs under certain conditions may, however, cause uptake and adverse effects greater than those induced solely by ionic silver species [10]. The transformations of AgNPs (e.g., oxidation to Ag^+ ions, formation of Ag_2S/Ag_2O precipitates, and reduction of ionic Ag^+ ions) modify their physicochemical properties and alter their transport and fate, resulting in complicated toxicological behavior [10,17]. At present, the question remains unanswered whether AgNP toxicity to aquatic organisms can be attributed to particulate silver or dissolved Ag^+ ions, or whether both are required [10]. Prior to assessing the environmental impacts of AgNPs discharged from widely used AgNP-containing products, we must first determine the two major silver species (Ag^+ ions and AgNPs) and elucidate their transformations in aquatic environments.

The concentrations of silver particulates and ionic Ag^+ in environmental compartments are believed to be on the order of several tens of nanograms per liter [10,18—21], obviously making their identification and quantification methodologically difficult and un-friendly. Although inductively coupled plasma mass spectrometry (ICP-MS) has excellent sensitivity for elemental determination, it requires appropriate sample pretreatment processes when used to identify trace Ag^+/AgNPs in aqueous environmental media [22,23]. Analytical methods based on field-flow fractionation [24,25], liquid chromatography [26—28], single-particle ICP-MS analysis [29—31], and cloud point extraction (CPE) [21,32—34] have been reported for studies of environmentally transformed AgNPs or/and Ag^+ ions. Nevertheless, several shortcomings remain: unavoidable sample dilution by the carrier solution or mobile phase, low analyte re-coveries, difficult instrumental and method optimization, and labor-intensive and time-consuming procedures. Devising efficient analytical strategies enabling rapid extraction/differentiation of AgNPs and their released Ag^+ ions from complicated aqueous environmental media should help us to assess AgNP transformations and their toxic responses toward aquatic organisms for the future development and use of AgNP-containing products.

Previously, we demonstrated that conventional knotted reactors (KRs) made from knotted arc-shaped PTFE tubing are capable of differentiating AgNPs and dissolved Ag^+ ions in biological tissues [35]. Additive manufacturing (three-dimensional printing; 3DP) is a powerful emerging technology for rapidly prototyping research equipment [36,37], allowing complex devices to be fabricated in multilayer working domains [38,39]. The conventional design of KRs has been introduced over two decades [40]. In this study, we employed a low-cost stereolithographic 3D printer to fabricate KRs containing the right-angled square flow channels. Unlike the flowing streams changing their directions smoothly in knotted arc-shaped PTFE tubing, shape-defined (e.g., square and rectangular) channels in the KR devices manufactured by 3DP technology can allow the changes of flow directions at an exactly designed angle, which should be able to significantly improve not only the reten-tion efficiencies but also the method's detection limits (DLs) when analyzing the AgNPs and Ag^+ ions. To facilitate the determination of environmental Ag^+/AgNPs, we evaluated these two silver species when stabilized by xanthan polysaccharides. After optimizing the KR design and the final online differentiation scheme, we applied the system practically to the quantitative differentiation of Ag^+/AgNPs in environmental media: the influent of a municipal wastewater treatment plant (WWTP) and municipal river water samples.

2. Materials and methods

2.1. Chemicals

AgNPs (658804; dispersed in pure ethylene glycol), xanthan gum from *Xanthomonas campestris* (G1253), and the powders used to prepare phosphate-buffered saline (PBS; P3813) were purchased from Sigma—Aldrich (MO, USA). A stock solution of Ag^+ ions (1000 mg L^{-1}) was obtained from Merck (Darmstadt, Germany). Nitric acid (6901) and ethylene glycol (9300) were purchased from J. T. Baker (NJ, USA). Standard solutions of Ag^+ ions and AgNPs were freshly prepared daily in the PBS solution containing 100 mg L^{-1} xanthan. The used cream UV-curable resin (BV-001, Rays Optics; US$138 per 550 g) comprised modified acrylate (10—25%), acrylate monomers (40—60%), acrylate oligomer (20—35%), and photo-initiators and additives (5—15%).

2.2. Apparatus and methods

A desktop stereolithographic 3D printer (MiiCraft®) was used to fabricate all-in-one KRs featuring (i) angle-defined square flow channels to improve the retention efficiencies of the two silver species and (ii) fittings for standard 10—32 flat-bottom male con-nectors at the channel inlets and outlets for convenient connection to a commercial flow injection analysis (FIA) interface. The KR de-vices were designed (SolidWorks 2013, Dassault Systèmes, Paris, France) in the form of a cuboidal block (Fig. 1A—C) in which the square tunnels (length × width × height: 1 mm × 1 mm × 3 mm) were aligned one-by-one with the overlapping of a 1 mm × 1 mm × 1 mm space (Fig. 1D and E). Because the flowing stream can return to its original direction after minimally passing the four units (Fig. S1, Supplementary Data), the retention efficacy of the four turns in the 3D printed KR was equal to that of one knot in a conventional KR homemade from knotted PTFE tubing. Fig. S2 (Supplementary Data) provides the dimensions of the designed KRs. The fabrication times for 128- and 768-turn KRs (100 μm per layer) were approximately 56 and 85 min, respectively, with total weights of 8.4 and 18.6 g, respectively. Detailed illustrations and descriptions of the operation of the MiiCraft® 3D printer are available elsewhere [39,41—43].

The KR sample pretreatment scheme involved four steps: sample loading, rinsing residuals in KR, elution of the analyte, and KR re-conditioning for the next loading. To automate these steps,