

The Effect of Photolysis on the Stability and Toxicity of Silver Nanoparticles

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ABSTRACT

This work investigates the effect of UV irradiation on the aggregation kinetics and toxicity of a 50 nm silver nanoparticle (AgNP) capped with two different stabilization agents (polyvinylpyrrolidone (PVP) and citrate). The particles were exposed to simulated sunlight by long wave ultra violet light (UVA) irradiation. The impact of the irradiation was investigated in solutions with and without 4 mg/L DOC. Field flow fractionation interfaced to inductively coupled plasma mass spectrometry (FFF-ICP-MS), provided a method to determine size and elemental composition of nanoparticles at environmentally relevant concentrations (parts-per-billion, $\mu\text{g/L}$). For PVP coated silver nanoparticles in the presence of DOC, photolysis appeared to create small nanoparticles as represented by growth in the void peak. Regardless of the stabilization coating, the standard zooplankton model, *Daphnia magna*, indicated that the toxicity of nanosilver was significantly reduced when exposed to UV light and was further reduced when exposed to UV light in the presence of DOC.

Keywords: silver nanoparticles, photolysis, *Daphnia magna*, field flow fractionation, dissolved organic carbon.

1 INTRODUCTION

Nanosilver is the most extensively used nanoparticle in current product applications. While a great deal of environmental health and safety research is underway, the majority of these studies address the fate and effects of nanosilver in pristine laboratory media. These conditions are unlikely to represent the fate and toxicity of nanosilver if released into the environment since they lack environmental ligands and wide spectrum natural sunlight. Many factors can affect particle stability, including the type of capping agent and the local aqueous conditions, such as pH, ionic strength and the presence of dissolved organic carbon (DOC). It is known from colloid science that the presence of DOC is likely to change the stability of nanomaterials, their interactions with other water constituents, and their transport behavior since the presence of DOC may lead to a surface coating of the nanoparticles with DOC molecules. Recent studies have provided evidence that while DOC increases the stability of nanosilver dispersions, the toxicity is reduced through

either complexation of metal ions or coating of particles [1]. An additional consideration is that silver nanoparticles suspended for an extended period of time in surface waters are subject to sunlight exposure, which may be an important factor affecting their stability, environmental fate and toxicity. It is altogether unknown how irradiation from wide spectrum sunlight (including the ultraviolet range) will influence the stability of coated nanosilver particle dispersions.

2 MATERIALS AND METHODS

2.1 Characterization of AgNPs.

Both polyvinylpyrrolidone (PVP) and citrate capped silver nanoparticles were used as supplied (Nanocompositx, San Diego CA). Primary particle size stated by the manufacturer was 49.1 +/- 4.5 nm for the citrate NanoXact product line and 48.3 +/- 3.7 nm for the PVP NanoXact product line as obtained from TEM imaging. The hydrodynamic diameter provided by the manufacturer was 54.3 nm and 57 nm for the citrate and PVP product line, respectively. The total concentration of the AgNPs was determined by graphite furnace atomic absorption spectroscopy after acidification to 3% nitric acid following standard USEPA method 7010 and utilized an ultrapure Palladium-Magnesium nitrate matrix modifier. Dissolved silver concentrations were measured from supernatant collection after 1 hr of ultracentrifugation at 100,000 x g [2]. A well characterized commercial source of HA (International Humic Substances Society- Suwannee NOM) was used in the experiments. Dissolved organic content was measured for a stock 10ppm NOM solution with USEPA method 9060 at 4.0%.

2.2 Field Flow Fractionation.

A Postnova Analytics F-1000 field flow fractionation system (Salt Lake City, UT) interfaced with a PerkinElmer Elan DRC II ICP-MS via a MiraMist pneumatic nebulizer was used for separation and detection of AgNPs. An Agilent 1100 variable wavelength detector was used to monitor UV absorbance for calibration with polystyrene standards as described previously [3]. Particles were

separated via interaction with a 1 kDa regenerated cellulose membrane in a mobile phase consisting of 0.01% each of NaN_3 and FL-70 surfactant in deionized water. The channel flow used was 1.0 mL/min with a cross flow of 0.75 mL/min. Silver signal was monitored for the ^{107}Ag isotope via ICP-MS with the plasma source operating at 1250 W and a nebulizer flow of 0.9 mL/min. Spectral data points were acquired at about 1 s^{-1} through the duration of the fractogram, typically $\sim 45 \text{ min}$.

2.2 Photolysis Experiments

Photolysis experiments were carried out in a fan cooled Rayonet Reactor RPR-100 (Southern New England Ultraviolet Company, CT). Because UVA is the primary component of UV light in the solar irradiation that can reach the earth surface, a light source in the UVA wavelength range was employed to mimic the UV fraction of the solar spectrum. Sixteen = 14W black lamps (SNE Ultraviolet Company $\lambda = 350 \pm 50 \text{ nm}$), provided approximately $0.05 \text{ mW/cm}^2/\text{nm}$ as stated by manufacturer. In house measurements with a handheld light meter (EasyViewTM 33, Extech Instruments, Nashua, NH) in the center for the reactor measured 9600 LUX for 1 hour, and was compared to the UVA intensity measured on a sunny summer day (1/12/2010) in Vicksburg, MS of 450 LUX for 1 hour. From this comparison it was determined that 1 hour of photolysis was equivalent to 24 hr exposure to sun. In each experiment 10 mL of the suspension was added to high purity MPM-214 quartz test tubes, placed on the merry-go-round at the center of the photoreactor, and slowly rotated to ensure uniform exposure.

2.3 Toxicity Bioassay Methodology

The acute toxicity (48-h) toxicity test method employing freshwater cladoceran *Daphnia magna* neonates (< 24-h old obtained from in-house cultures) was selected due to the known rapid onset of mortality caused by dissolved and AgNP [2]. Exposures were conducted at $25 \pm 1 \text{ }^\circ\text{C}$ without a feeding ration in basic accordance with standard guidance [4]. Moderately-hard reconstituted water (MHW) was spiked with AgNP (photolysed or non-photolysed) and serially diluted (0.5) using MHW as the diluent and negative control. In NOM experiments, the DOC concentration was kept constant as silver was diluted. Survival was assessed after 2, 24, and 48-h post exposure. Total and dissolved silver were sampled at 1 and 48-h into the exposure as previously described [2]. No observable effect concentrations (NOEC), lowest observable effect concentrations (LOEC), and lethal median concentrations (LC50) were determined from measured concentrations using ToxCalc Software v5.0 (Tidepool Scientific Software, McKinleyville, CA, USA). LC50 values with non-overlapping 95% confidence intervals were considered statistically significant.

3 RESULTS

The stock concentrations for the PVP and citrate AgNP were measured at 22.0 mg/L and 18.0 mg/L, respectively, as confirmed by graphite furnace analysis. Using ultracentrifugation, the dissolved fraction of Ag ions in the stock solutions were measured at 16.6 wt% (3.6 mg/L) for the PVP AgNP and 1.4% (0.25 mg/L) for the citrate AgNP. FFF-ICP-MS fractograms for 200 $\mu\text{g/L}$ solutions of the AgNPs as a function of different diluents and capping agents are shown in Figure 1. The diluents chosen were DI water, MHW, and a 4 mg/L DOC solution in the MHW. The fractograms show citrate AgNP diluted in DI water with an average particle size of 58 nm as sized via FFF methodology with a narrow size distribution as compared to the PVP AgNP with an average particle sized of 69 nm and a particle distribution slightly skewed to larger particles. When the AgNP were prepared in the MHW solutions at the same concentration the particles became unstable and fell out of solution at 200 $\mu\text{g/L}$ as illustrated by loss in peak intensity regardless of capping agent. When the AgNPs are introduced to a MHW/NOM solution the particles are more stable with less loss in peak intensity and a slight decrease in particle size.

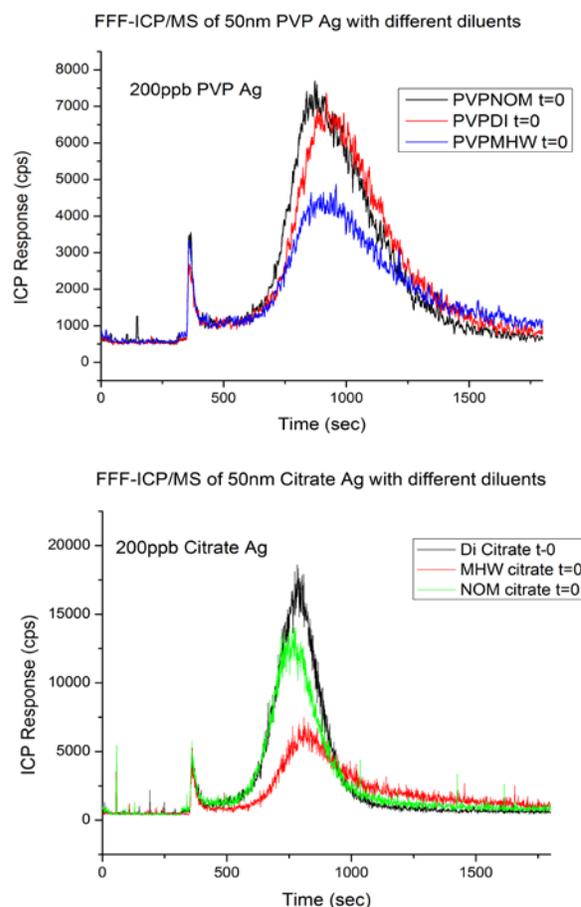


Figure 1. FFF-ICP/MS Fractograms for citrate and PVP stabilized AgNPs as a function of different diluents.

Representative FFF-ICP/MS fractograms for 200 µg/L suspensions of the AgNPs as a function of photolysis are shown in Figure 2, for DI water solutions and the MHW/NOM blend. After photolysis of the AgNP in DI water the particle size is reduced regardless of capping agent. In the case of the MHW/NOM blend there is a decrease in particle size, but also an unexplained increase of the void peak. The growth in the void peak with increasing photolysis time was consistent regardless of capping agent. The void peak represents material that is larger than the 1kDA membrane (impurities) used in the experiments or particles not retained under the cross flow field conditions during the relaxation period due to inadequate field strength. In an “ideal case” all of the dissolved Ag⁺ should pass through the membrane and not be associated with the void peak and the void peak should disappear if the field is increased to allow for all particles to have substantial time to interact with the membrane. Experiments were performed under increasing field strength conditions that showed the void peak could be eliminated completely for solutions that were not photo-treated. However, the void peak remained for all systems containing DOC treated with photolysis. This result suggests the photolysis produces smaller silver particles or complexed particles not present in the starting solution.

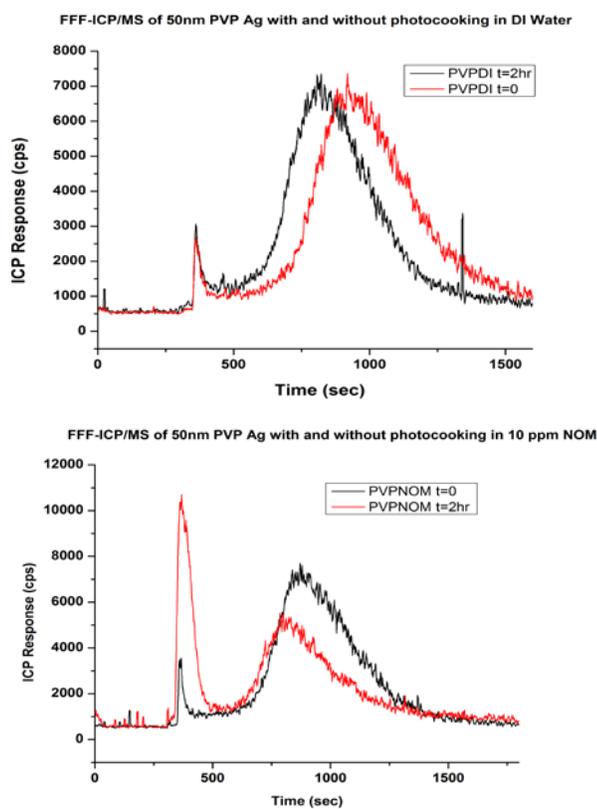


Figure 2. FFF-ICP/MS Fractograms for PVP stabilized AgNPs with and without NOM before and after photolysis.

To discern between the possibility of complexation versus small particle growth photolysis experiments were performed on a silver nitrate solution void of nanoparticles. Figure 3 shows FFF-ICP/MS fractograms for AgNP growth from solutions of 1.8 µg/L silver nitrate in 10 mg/L NOM (4mg/L DOC) after a 2 hours of photolysis. Particles were observed after photolysis in the presence of DOC. Before photolysis, no peaks were discernable with FFF-ICP-MS. The FFF fractograms provide evidence for the creation of very small nanoparticles in the void peaks.

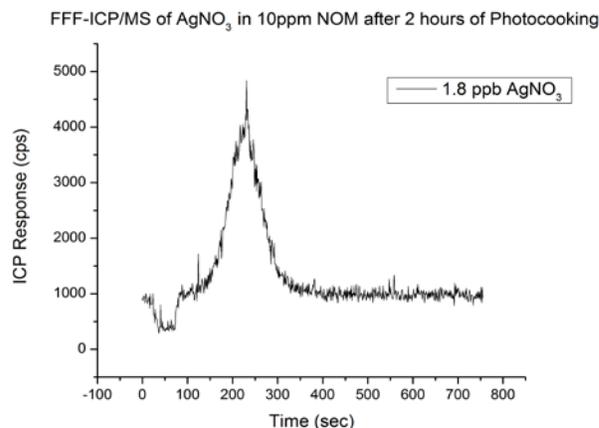


Figure 3. FFF-ICP/MS Fractogram of silver nanoparticles produced from photocooking silver nitrate solutions in the presence of 10ppm NOM (4mg/L DOC).

Figure 4 provides a dose-response curve for the exposures of *D. magna* to different treatments of the PVP AgNP. Toxicity was assessed on the basis of both total and dissolved silver. The baseline LC50 for the unmodified PVP AgNP was 9.7 (7.7 - 12.2) µg/L, expressed as total measureable silver. This toxicity was statistically significantly reduced as the DOC concentration was increased from 0 to 4 mg/L (Table 1). Photolysing the AgNP in the absence of DOC (0 mg/L) also significantly reduced its toxicity. The largest reduction in toxicity was observed when the AgNP was photolysed

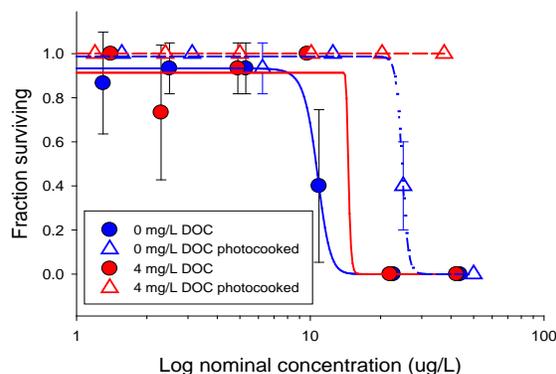


Figure 4. Dose response curves for *Daphnia magna* exposed to different AgNP treatments for 48-hours.

in the presence of 4 mg/L DOC, with no mortality observed up to 37 µg/L. This was at least a 3.8-fold reduction relative to the baseline toxicity.

Calculation of LC50 values expressed by the dissolved silver concentration for the non-photolysed PVP AgNP in presence and absence of DOC and the photolysed silver in the absence of DOC fell within the range of 1.3 to 2.5 µg/L. These LC50 values are very comparable to the range of toxicity previously reported for dissolved silver as AgNO₃ of 0.6 – 2.9 µg/L [2], suggesting that the majority of the acute toxicity of these AgNP suspensions could be explained by the dissolved fraction. However, for the PVP AgNP photolysed in the presence of DOC, no toxicity was observed up to a dissolved concentration of 8.6 µg/L; this concentration is three times greater than the highest reported LC50 for dissolved silver. This suggests that the 8.6 µg/L dissolved silver is either no longer bioavailable to the *D. magna* due to enhanced complexation with the DOC or that there was a fundamental change to the dissolved silver fraction, such as creation of very small AgNP particles (< 5 nm) that ultracentrifugation was incapable of removing from the supernatant. The FFF fractograms (Figure 2) provide evidence for the creation of very small AgNP in the void peaks. In any case, these data clearly indicate that the toxicity of the AgNP suspension was substantially reduced due to environmentally relevant mitigating factors (sunlight, DOC).

Table 1. Summary of 48-h *Daphnia magna* lethal median concentrations (LC50) generated from exposure to the PVP nanosilver. 95% confidence limits are provided in parentheses.

| Treatment | 48h LC50 | 48h LC50 |
|-----------------------------|--------------------|-------------------|
| | Total Ag | Dissolved Ag |
| PVP-nAg | 9.7 (7.7 - 12.2) | 1.8 (1.5 - 2.3) # |
| PVP-nAg (4 mg NOM/L) | 14.5 (14.2 - 14.9) | 2.5 (2.4 - 2.5) # |
| PVP-nAg cooked | 16.2 (13.2 - 20.0) | 1.3 (1.1 - 1.6) |
| PVP-nAg cooked (4 mg DOC/L) | > 37.4 | > 8.6 |

LC50 was estimated from the dissolved fraction of the high concentration due to no detects

4 CONCLUSIONS

In conclusion, we determined that AgNPs undergo some aggregation with photolysis resulting in a reduction in toxicity. A further reduction in toxicity was observed when photolysis occurred in the presence of Natural Organic Matter. It was suggested that the dissolved Ag ions present in solution react in the presence of DOC to form small AgNP that result in a reduction in toxicity. The

significance of these findings is that while nanosilver is toxic to aquatic organisms, its toxicity is likely to diminish substantially when subjected to inevitable environmentally relevant mitigating factors such as dissolved organic matter and sunlight.

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