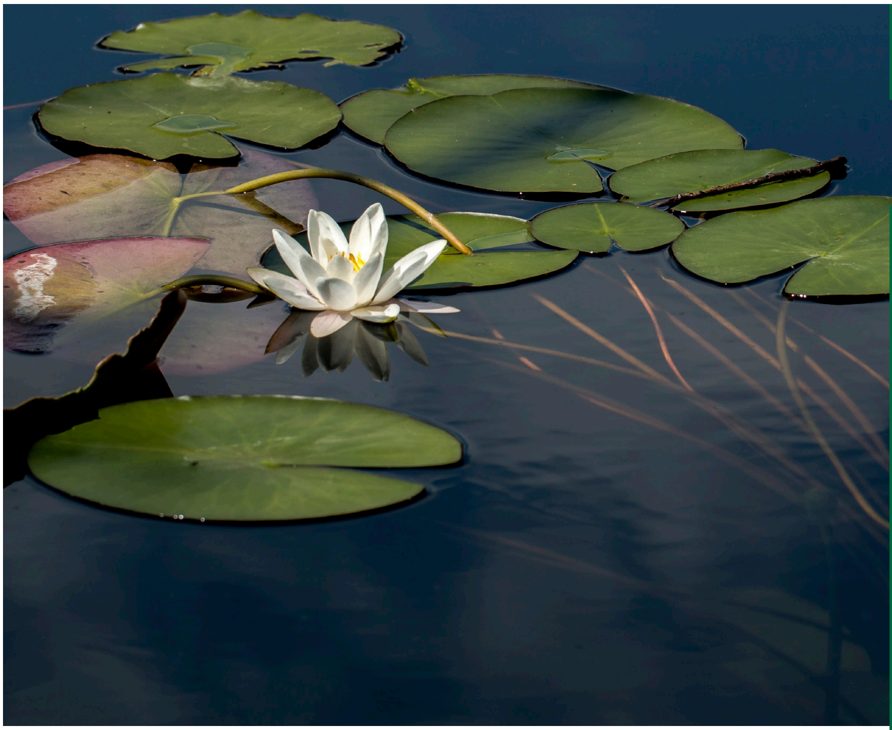

APPLICATION NOTE

ICP - Mass Spectrometry

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Transformations of Gold-Silver Core-shell Nanoparticles in Exposure Media Measured by SP-ICP-MS

Introduction

Engineered nanoparticles (ENPs) are widely used in both industrial processes and consumer products, and, therefore, have well-controlled properties and characteristics for their specific applications. Through their use, ENPs will inevitably make their way into the environment. However, it can be expected that that ENP concentrations in the environment will be much lower than those produced and used in industry. The fate and behavior of ENPs in the environment is largely unknown, mostly due to a combination of the complexity of environmental systems along with the tailored characteristics of ENPs. However, ENPs can undergo numerous transformations: aggregation, dissolution, chemical interactions with salts/metals in the media, and physical interactions are just a few of the possibilities.

Current research into environmentally-induced ENP transformations is limited by analytical instrumentation capabilities, including detection limits, sample preparation, and/or time required for data acquisition/analysis. As a result, a multi-method approach is usually required to measure size, aggregation/dissolution kinetics, and chemical alterations; commonly-used techniques include electron microscopy, optical microscopy/spectroscopy, and separation of particulate sizes and ionic fractions. Most of these methodologies require ENP concentrations of at least 25 - 50 µg/L, with the majority of studies being done at 100 - 1000 µg/L. These concentrations are much greater than those typically predicted to be in environmental systems, which for most ENPs is less than 1 µg/L.

Studying ENPs at higher concentrations drastically alters factors that affect transformations, including increased probability of ENP-ENP interactions, lower ENP-to-salt ratios (ENP:salt), and lower ENP-to-natural-organic-matter ratios (ENP:NOM). In fact, previous studies have shown that ENP transformations at 1000 µg/L are different to those at 100 µg/L.[1, 2]

This work shows that single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) is able to overcome the particle concentration limitations of other techniques, almost eliminate sample preparation, and dramatically reduce the acquisition time required to accurately measure the size, size distribution, and particle number concentration (part/mL) of ENPs at more environmentally relevant concentrations (less than 1 µg/L).

SP-ICP-MS is used to study the transformations of ENPs under controlled conditions (meant to mimic toxicological and environmental systems) at time points over a 48-hour period (a typical acute exposure period). This fundamental understanding and methodology can be applied to real-world samples.

Silver (Ag) ENPs were chosen for this case study due to their use in many commercial products, increasing their likelihood of entering environmental systems. They have been shown to be highly toxic, especially in smaller organisms such as bacteria and algae.[3, 4] They are known to react in the presence of sulfide, NOM, chloride, and exposure to natural light. Recently, it has also been shown that Ag NPs can be formed in solutions containing ionic Ag and NOM when exposed to light and heat,[5, 6] thus adding extra complications to the measurement of Ag ENP transformations. Because of the speed and complexity of these transformations, it was decided to use core-shell particles consisting of a less reactive core material (Au) with an Ag shell.

Experimental

Standards, Samples, and Sample Preparation

All exposures were performed with gold-silver core-shell nanoparticles (denoted as Au@Ag) which consist of a 30 nm gold (Au) core and a 15 nm silver (Ag) shell to give a final particle diameter of about 60 nm (Nanocomposix™, San Diego, California, USA). These particles were chosen so that the internal Au NP could be used to monitor the complicated Ag transformations. This difference in reactivity allows a more accurate characterization of the processes occurring in solution.

Transport efficiencies were determined with 30 nm Au nanoparticles (NIST™ 8012, Gaithersburg, Maryland, USA) at a concentration of 100,000 particles/mL. Dissolved calibration standards for both Au and Ag (1, 2, 3 µg/L) were made by serial dilutions of 1000 mg/L stock solutions (Fisher Scientific™). All standards and blanks were made fresh before use and were matrix matched with the exposure samples.

Exposure studies were carried out in three different media: deionized water (DIW), moderately hard water (MHW), and MHW with the addition of 2.5 mg/L fulvic acid (FA); the specific characteristics of these waters are shown in Table 1. The MHW was synthetically produced using EPA guidelines (all chemicals from Fisher Scientific™). These matrices were chosen to cover a variety of situations: a control (DIW), a typical drinking water (MWH), and a sample which more closely mimics environmental surface waters (MHW-FA).

To ensure no particle carryover, a three-minute wash cycle followed each sample: 2 minutes of 1 % aqua regia (to dissolve any remaining ENPs) and 1 minute of ultrapure water to wash out the acid. The size and concentration of freshly made Au@Ag solutions and Au 30 nm NIST particles were measured before and after each time point and periodically between exposure samples to ensure that standard solutions were still returning the correct results.

Table 1. Characteristics of Water Samples.

Media	Acronym	pH	Composition
Deionized Water	DIW	5.3	---
Moderately Hard Water	MHW	7.4 - 7.8	Sodium hydrogen carbonate (96 mg/L)
			Magnesium sulfate (60 mg /L)
			Calcium bicarbonate (60 mg /L)
			Potassium chloride (4 mg /L)
Moderately Hard Water with Fulvic Acid	MHW-FA	7.4 - 7.8	Sodium hydrogen carbonate (96 mg/L)
			Magnesium sulfate (60 mg/L)
			Calcium bicarbonate (60 mg/L)
			Potassium chloride (4 mg/L)
			Suwannee River fulvic acid (2.5 mg/L)

The studies were carried out with particle concentrations between 10 thousand (K) and 44 million (M) parts/mL (0.01 – 46 µg/L of Ag). Solutions were subjected to different conditions (as shown in Table 2) for 48 hours. Conditions were controlled using an Innova® 44 incubator shaker (New Brunswick Scientific™), fitted with a broad spectrum UV/Vis (Ultra Violet/Visible) lamp. Aliquots of the samples were removed at time intervals of 0, 12, 24, and 48 hours during the exposure and analyzed immediately without any extra sample preparation to ensure as little change as possible from the original sample. The Ag signal was measured, followed by the Au signal as the Ag component of the ENP is more susceptible to changes than the Au.

Analytical Conditions

All SP-ICP-MS data was acquired with a PerkinElmer NexION® 350D ICP-MS operating in single particle mode with the Syngistix™ Nano Application Software Module using the conditions specified in Table 3. All samples were run twice: once while monitoring Ag and once for Au. Each analysis provided the following information: particle size, particle concentration, most frequent size, mean size, and dissolved (ionic) concentration.

A core-shell particle in SP-ICP-MS will be measured as two different diameters (one for the Ag signal and one for the Au signal), neither of which will read as the expected total size of the ENP. In this case, the Au particle size will be seen as about 30 nm, as expected from the manufacturer. But the particle size of the Ag layer will be seen as smaller than 60 nm. A simple calculation can be performed to determine the number of atoms[7] expected in the 15 nm Ag shell, which is equivalent to a diameter of 57 nm. An important fact to keep in mind is that by removing 3 nm of Ag atoms from the surface of a 60 nm particle, there are enough atoms to make a 30 nm Ag ENP. This fact can imply that an increase in particle number concentration is possible, indicating the dissolution and re-precipitation of silver in the studied media.

Table 2. Exposure Test Conditions.

Set	Test	Media	Particle Concentration	Temperature (°C)	Light Exposure Time (hours)	Exposure Duration
1	1	DIW, MHW, FA	100 K	15	0	48
	2	DIW, MHW, FA	100 K	15	16	48
	3	DIW, MHW, FA	100 K	22	0	48
	4	DIW, MHW, FA	100 K	22	16	48
2	5	MHW	10 K, 50 K, 100 K, 250 K, 22 M, 44 M	22	16	48
	6	FA	10 K, 50 K, 100 K, 250 K, 22 M, 44 M	22	16	48

K=thousand; M=million

Table 3. NexION 350D ICP-MS Instrumental Conditions.

Parameter	Value
Sample Uptake Rate	0.31 mL/min
Nebulizer	Meinhard™ glass concentric
Spray Chamber	Glass cyclonic
Injector	2 mm ID, quartz
RF Power	1600 W
Dwell Time	100 µs
Electronics Settling Time	0 µs
Data Acquisition Time	60 – 300 sec
Transport Efficiency	9-11 %
Analytes	^{107}Ag, ^{197}Au
Tubing	Orange/green (0.38 mm id)
Spray Chamber	Cyclonic
Cones	Platinum
RF Power (W)	1400

Results and Discussion

In all studies, the ENPs were maintained in controlled environments for 48 hours, with aliquots withdrawn at 0, 12, 24, and 48 hours for analysis. By monitoring the particle size and number concentration (for both Ag and Au) over time, the degree of ENP transformation (aggregation, dissolution, and dissolution/re-precipitation) can be observed. An increase in both Au and Ag particle size, with corresponding decrease in particle number, indicates aggregation, while a decrease in Ag particle size and stable Au particle size indicates dissolution. An increase in Ag diameter while the Au diameter remains unchanged, but a decrease in Ag and Au particle numbers indicates a more complicated transformation, such as ENP aggregation alongside Ag dissolution and reprecipitation onto the NPs in suspension.

Two sets of conditions were used. The first (experiments 1-4 in Table 2) were chosen to simulate some standard toxicological test conditions[3, 8] and synthetic surface waters, which includes temperature, UV exposure, and the presence of natural organic matter (NOM). The second set looked at the effect of ENP concentration on transformations (Table 2, experiments 5-6). The highest concentration is similar to that used in recent ENP transformation exposures,[1] and the lower end is in the range expected to be found in the environment.[9]

The first study revealed that the different conditions affected which processes were most prevalent in Ag ENP transformations. Figure 1 A-D (A: 15 °C no light exposure; B: 15 °C 16 hrs light exposure; C: 22 °C no light exposure; and D: 22 °C 16 hrs light exposure) displays the % change of the ENP diameter from 0 to 48 hours. (The results at 12 and 24 hours are trending in the same direction as those for 48 hours, and are not included here for clarity). A negative change indicates the particle decreasing in diameter, while a positive change indicates an increase in particle diameter.

Figure 1 shows the % change in particle diameter after 48 hours for a high and low concentration of the NP suspensions in all three media and under four different conditions (described in Table 2). At low temperatures (Figure 1 A and B), there is very little difference in the ENP between the light and dark conditions, with the exception of FA at the lower concentration when exposed to light. In general, there is a reduction in Ag size, while there is no significant change in the Au size for both light and dark. There is also no significant change in the particle number from 0 to 48 hours for both the Ag and Au signals. The evidence suggests dissolution of the outer Ag shell. In the FA suspensions, the same trends are observed for both the concentrations in the dark and the higher concentration when exposed to light. But there is a slight increase in both the Ag and Au size for the lower concentration with light, suggesting that the light is causing an interaction with the ENPs and resulting in increased stability, although some aggregation is still observed.

More drastic changes were observed at a higher temperature, both at low and high concentrations. Figure 1C shows the changes seen at 22 °C with no light exposure. For the higher concentration, a positive % change can be seen for both the Ag and Au NP diameter (there is a corresponding negative change in the particle number for both Ag and Au signals) for the DIW and MHW media. This suggests that aggregation is occurring in both of these suspensions. For the high concentration FA suspension and all the suspensions for the low concentrations, there is a positive change for the Ag size and no real change for the Au size. Along with this observation, the Ag particle numbers are also increasing, while the Au particle number remains stable. This suggests a complicated process is occurring, where dissolution is taking place, and the ions are either reprecipitating (i.e forming new Ag particles) or causing a ripening effect on the outside of existing particles in suspension, causing an Ostwald ripening effect.

At 22 °C, the presence of light (Figure 1 D) causes a dramatic change. At the higher concentrations, there is an increase in ENP diameter for all three suspensions in both the Ag and Au case. There is also a decrease in particle number for these suspensions with the most significant decrease occurring in the DIW experiment. Thus, aggregation is occurring in all samples to some extent. However, there is a "protective" effect occurring in both the MHW and FA cases. For the lower concentrations, there is no significant % change in the Au NP diameter but a % decrease in the Ag diameter. However, there is an increase in the number of Ag particles / mL with the least amount of change for the DIW suspension. This suggests that the heat and light are causing the ENPs to dissolve rapidly, but in the presence of MHW and FA, there is some reprecipitation occurring.

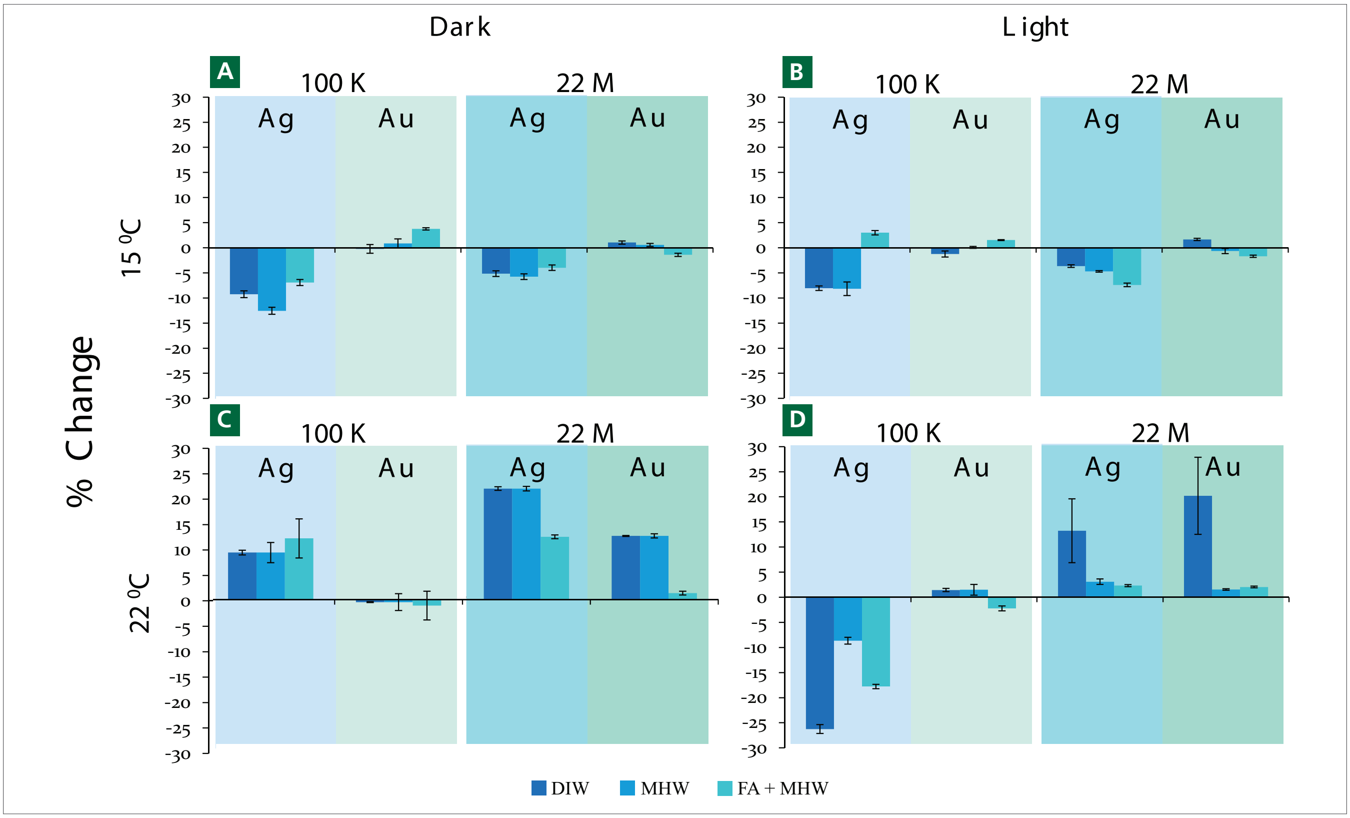


Figure 1. Effect of light, particle concentration, matrix, time, and temperature on ENP transformations for A) 15 °C no light exposure, B) 15 °C 16 hrs light exposure, C) 22 °C no light exposure and D) 22 °C 16 hrs light exposure.

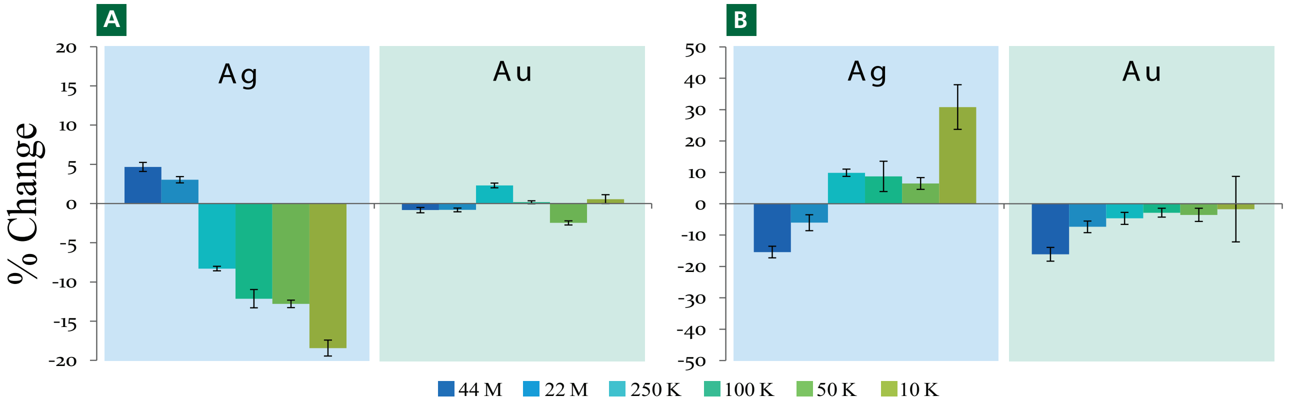
From this study, we can see that temperature, exposure to light, presence of NOM, and ENP concentration all have a significant effect on the types of ENP transformations. At both temperatures, ENPs in all waters behave similarly in the dark, whereas there is a significant difference between the high and low concentrations at 22 °C with light exposure.

The second set of studies (Experiments 5-6 in Table 1) explored the effect of particle concentration on ENP transformations at 22 °C, with 16 hours of UV exposure over 48 hours. Figure 2 shows the % change from 0 to 48 hours for (A) the particle diameter and (B) part /mL for the ENPs in MHW (for comparisons, the actual measured values can be seen in the corresponding Table, C). The graphs show the results for six concentrations ranging from 44 M to 10 K part / mL.

It can be seen that at 44 M and 22 M, there is an increase in both the Ag and Au particle diameter and a corresponding decrease in part/mL. The number of part/mL drops at the same rate for both the Ag and Au signals; since these are core-shell particles, it can be assumed that the particles are undergoing aggregation due to their equivalent loss. At concentrations of 250 K and below, the Ag ENP size decreases, while their concentration increases. For these sample conditions, the Au particle diameter and number do not change significantly over 48 hours. These observations suggest that the Ag layer is not only dissolving from the particle, but also reprecipitating as other particles, possibly as a silver complex (sulfide, oxide or chloride). The % change in Ag particle diameter and part/mL becomes larger as the original concentration of particles in suspension is decreased, meaning that the drop in original concentration causes the dissolution and reprecipitation rate to increase over 48 hours.

Figure 3 shows the % change after 48 hours for the particle diameter (A) and ENP concentration (B) along with the measured values (C) for original concentrations between 44 M to 10 K part/mL in FA media. The highest concentrations of 44 M and 22 M part/mL show a positive change in particle diameter for the Ag signal only with a corresponding negative change in particle number for both Ag and Au. The stability in the Au particle diameter along with the growth in Ag diameter suggests that the Ag layer is growing due to the deposition of Ag ions onto the surface or onto fulvic acid molecules that are bound to the ENP surface. This, along with the decrease in part/mL for both Ag and Au signals, implies that not only are the particles aggregating and settling out of the suspension, but they are also dissolving, and the excess Ag ions are depositing onto the suspended particles. This highlights the necessity of the core-shell particle, as just looking at the Ag data would lead to the conclusion that the particles are aggregating.

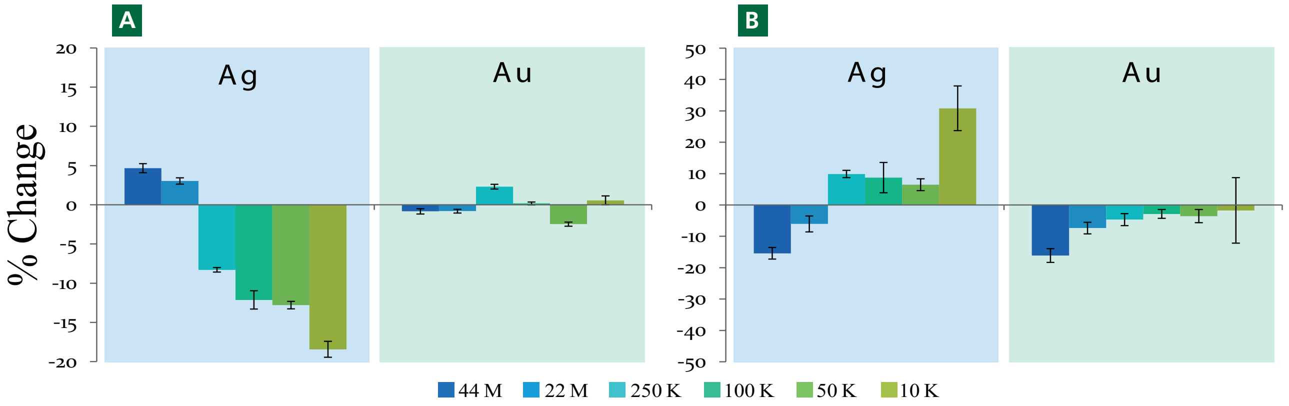
At concentrations below 250 K, the Au particle diameter and particle number are stable. But there is a decrease in the Ag particles size and increase in the Ag particle number suggesting dissolution and precipitation of the ENPs that is increasing in rate as a function of particle number.



C

		Original Particle	44 M	22 M	250 K	100 K	50 K	10 K
Measured Au size (nm)		31.0	33.2	32.0	31.1	31.3	31.1	31.0
Measured Ag size (nm)		57.7	62.7	61.0	55.3	54.7	54.1	52.6
Calculated total particle size (nm)		60.6	65.7	63.8	58.4	58.0	57.4	56.0
Particle Conc (part/mL)	Ag	---	32.3 M	17.6 M	259 K	115 K	62.1 K	21.1 K
	Au	---	31.1 M	19.0 M	232 K	102 K	49.9 K	9.7 K

Figure 2. The % change of A) particle diameter and B) particle number concentration of ENPs in MHW after 48 hours. The corresponding table (C), shows the actual measured values for the particle diameter and concentration, as well as the calculated total diameter of the ENP.



C

		Original Particle	44 M	22 M	250 K	100 K	50 K	10 K
Measured Au size (nm)		31.0	30.9	30.9	31.9	31.3	30.4	31.4
Measured Ag size (nm)		57.7	60.2	59.2	52.7	50.5	50.1	46.9
Calculated total particle size (nm)		60.6	62.8	61.9	56.4	54.3	53.6	51.2
Particle Conc (part/mL)	Ag	---	37.6 M	20.6 M	276 K	111 K	55.5 K	20.6 K
	Au	---	37.7 M	20.6 M	238 K	96.0 K	47.4 K	10.6 K

Figure 3. The % change of A) particle diameter and B) particle number concentration of ENPs in FA after 48 hours. The corresponding table (C) shows the actual measured values for the particle diameter and concentration as well as the calculated total diameter of the ENP.

Table 4. Number of Fulvic Acid Molecules per Nanoparticle.

Particle Concentration			Number of FA Molecules	
Particles/mL	Ag (µg/L)	Au (µg/L)	Molecules/nm^2 of Particle Surface Area	Number of FA Molecules / NP
22,000 M	23,000	6,000	8	85,511
44 M	46	12	3,780	4.28E+7
22 M	23	6	7,561	8.55E+7
250 K	0.261	0.068	665,355	7.53E+9
100 K	0.104	0.027	1,663,386	1.88E+10
50 K	0.052	0.014	3,326,773	3.76E+10
10 K	0.010	0.003	16,633,863	7.88E+11

It is important, when thinking about interactions of ENPs with NOM, to consider the ratio of ENP surface area to NOM molecule concentration. For the calculations made in Table 4, we have assumed the molecular mass to be between 800 – 1000 Da taken from several papers as the most frequent size of Suwannee River fulvic acid in surface waters (which can change with pH and ionic concentration)[10, 11] and a concentration of 2.5 mg/L of FA.

In Table 4, it can be seen that the number of available molecules of FA per nm^2 of particle is dramatically different from the stock suspension (8 molecules/nm^2) for the concentrations studied. At the most dilute suspension in this study (10K particles / mL), there are over 16,000,000 FA molecules available per nm^2 of ENP. We have shown here that when the ENP concentration is decreased with respect to a simulated surface water containing a fixed amount of FA, there is a dramatic effect on the ENP transformations going from a stabilizing effect at higher concentrations to accelerating dissolution at lower concentrations. This ratio is an important factor to consider when conducting transformation experiments.

Conclusions

SP-ICP-MS has been used to help understand ENP transformations in exposure and environmental media. Using this methodology, it is possible to obtain information on ENP size and particle number concentration, among other properties, at environmentally relevant concentrations, about 100 times less concentrated than in previous studies. In addition, SP-ICP-MS can acquire this information in 1 - 5 minutes per sample, rather than hours/days to obtain the same data using a combination of electron microscopy and optical spectroscopy techniques. However, for very complex transformations such as particle shape and whether complexes are formed with sulfides, oxides, and/or chlorides, additional data are needed.

This work has demonstrated that FA helps stabilize higher concentrations of ENPs at lower temperatures in the presence of light. However, it appears that at lower ENP concentrations, light and higher temperatures seemingly accelerate dissolution and some re-precipitation. There are differences between all high and low ENP concentrations pointing towards ENP:salt concentration and ENP:NOM concentration being key ratios when looking at NP transformations.

The advantages of SP-ICP-MS are obvious, and incorporating core-shell particles (where the core is stable and less likely to dissolve than the coating) makes analysis of the more reactive outer shell transformations easier to interpret. It is difficult to point out the nature of the silver species formed (sulfides, oxides, chlorides, among others) without a multi-method approach in which ICP-MS and SP-ICP-MS are essential parts.

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Consumables Used

Component	Description	Part Number
Sample Uptake Tubing	Green/orange (0.38 mm id) flared PVC	N0777042
Drain Tubing	Grey/grey (1.30 mm id) Santoprene	N0777444
Gold Nanoparticles	30 nm spherical gold nanoparticles in water	N8142300
Gold Standard	1000 mg/L aqueous gold standard, 125 mL	N9303759
Silver Standard	1000 mg/L aqueous gold standard, 125 mL	N9300171

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