THE INFLUENCE OF MAJOR FRESHWATER IONS ON THE BIOAVAILABILITY OF SILVER NANOPARTICLES TO DAPHNIA MAGNA

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To: Graduate Program Committees, Huxley College

Total required funding for project: $1,162.50
Amount requested from Graduate Program Committees: $1,162.50
Project Duration: May 2017 – September 2017

Claire Walli  4/14/2017
Applicant signature  Date

Approved by:

[Signature]
Print Thesis committee chair name here  - Ruth Sofield

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Print Thesis committee member name here  - Steven Emory

[Signature]
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Print Thesis committee member name here  -
Background

Silver nanoparticles (AgNPs) are increasing in presence in commercial and medical products due to their bactericidal properties. When materials containing AgNPs are washed, traces of them are transported through wastewater treatment facilities and into the environment. Once there, they may have toxic effects on aquatic organisms.

In freshwater ecosystems, many organisms are extremely sensitive to the water chemistry of the environment in which they live. Chemicals present can react with sensitive areas on the organism where important biochemical reactions occur, such as the gills on a fish. In aquatic toxicology, these reactive areas are referred to as the biotic ligand and have been well studied as the locations where metals in solution bind to and cause toxicity. The mechanism of toxicity at the biotic ligand can be modeled based on the water quality parameters present, some of which exacerbate toxicity, while others protect against it. These water quality conditions affect the bioavailability of metals, which is to say, whether or not they are available to interact with an organism and have a toxic effect. Major cations in freshwater, such as Na and Ca, compete with the metal ions for binding sites at the biotic ligand thereby reducing the concentration of metal at the site of toxicity, consequently decreasing the toxic effect. The bioavailability of silver ions (Ag+) that have dissociated from AgNPs can be modeled using the biotic ligand model (BLM).

Freshwater ions have also been proven to strongly impact AgNP behavior. The surfaces of AgNPs are extremely reactive to chemicals in their surrounding environment. They are highly subject to chemical oxidation and reduction, dissolution of Ag+ into solution, aggregation of particles clumping to one another, sedimentation of larger particles out of solution, and other physical and chemical transformations. The presence of freshwater cations have been proven to predictably cause AgNP aggregation by overcoming the natural repulsion that nanoparticles have for one another. Alternatively, the types of anions present can further change those aggregation behaviors; Baalousha et al. found that the aggregation rates of AgNPs changed when the same concentration of Na+ was paired with Cl-, NO3- or SO42-. This finding supported Li et al., which observed different rates of aggregation when AgNPs were suspended in solutions of NaCl or NaNO3 of identical concentrations. Aggregation behaviors have been proven to affect the dissolution behavior of Ag+ release from the surface of AgNPs.

Changes in particle behavior have been proven to alter the toxicity of AgNPs, however the mechanism of toxicity is still debated. Dissolution of Ag+ from the AgNP surface is argued to
be the toxic mechanism of action of AgNPs\textsuperscript{22-25}; the toxicity of ionic silver is well studied and can be explained using the BLM. However, the toxicity of AgNPs is not entirely predictable by the BLM and the reason why is still being debated\textsuperscript{11}.

**The purpose of my research** is to observe the effects of individual freshwater ions on the toxicity of AgNPs to the freshwater daphnid, *Daphnia magna*. My project design aims to explain whether differences in toxicity can be explained by changes in the behavior of the nanoparticles or are instead due to protection against or exacerbation of toxicity of dissolved Ag\textsuperscript{+} at the biotic ligand.

**Materials and Methods**

**Experiment Design**

My study will be centered around acute *Daphnia magna* toxicity tests following ATSM\textsuperscript{26}. Each test will incorporate a concentration gradient of AgNPs in order to develop a concentration-response curve. These will be performed in synthetic freshwater with different ionic compositions. Each concentration of AgNPs will be replicated three times in each test, and each test will be replicated twice. Mortality will be the measured effect and the concentration that kills 50\% of the test organisms (LC50) will be determined with the best fit model for the concentration-response curves using the drc package in R.

The synthetic freshwater will follow the synthetic moderately hard water (MHW) recipe in USEPA\textsuperscript{27}, which includes set concentrations of KCl, NaHCO\textsubscript{3}, CaSO\textsubscript{4}, and MgSO\textsubscript{4}. Each test will then include additions of either a determined “high” or “low” concentration of NaCl, CaCl\textsubscript{2}, or NaNO\textsubscript{3}. These particular salts have been selected to compare cation charge against different concentrations of Cl\textsuperscript{-} and NO\textsubscript{3}\textsuperscript{-}, common anions in environmental freshwaters. These high and low concentrations will be reflections of environmentally realistic concentration ranges. Combinations of the additional salts will be as represented in Table 1 to create eight different water conditions.

Using Principal Components Analysis (PCA), existing differences in toxicity between waters with different combinations of major ion concentrations should be attributable to the particular changes in concentration of a specific ion. These differences should be able to be compared against observed particle behaviors to determine whether differences in toxicity correlate with those behaviors or whether they agree with ionic silver toxicity modeled by the BLM.
Table 1. Schematic of salt additions to USEPA\textsuperscript{27} MHW recipe. Each row is an experimental condition where the salts are added to the MHW synthetic freshwater. (+) indicates the determined “high” addition of that salt and (−) indicates the determined “low” addition of that salt.

<table>
<thead>
<tr>
<th></th>
<th>NaCl</th>
<th>CaCl\textsubscript{2}</th>
<th>NaNO\textsubscript{3}</th>
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Materials

Citrate-capped AgNPs will be synthesized using the method in Lee and Meisel\textsuperscript{28} using AgNO\textsubscript{3} and 1\% sodium citrate. Daphnids will be purchased from Aquatic Biosystems (Fort Collins, CO) in three batches: the first for range-finding experiments to find appropriate AgNP concentrations in each synthetic freshwater, the second and third shipments will be for the LC50 tests.

Characterization of AgNPs

Samples for physical and chemical characterization will be conducted at the AgNP concentration equal to the LC50 in each of the eight water conditions. Analytical measurements will be taken 48 hours after AgNP addition. A minimum of two replicates of each LC50 concentration will be measured with each characterization.

Particle concentration and particle size distribution will be measured on the inductively coupled plasma mass spectrometer (ICP-MS) using single particle ICP-MS according to Pace et al.\textsuperscript{29}. Dissolved Ag\textsuperscript{+} ion content will be measured by ultracentrifuging AgNP samples and measuring the supernatant on the ICP-MS according to Kennedy et al.\textsuperscript{30}. Electrolyte concentrations may be measured using ion chromatography according to Mclaughlin and Bonzongo\textsuperscript{14} and ICP-MS.
**Anticipated Results**

I expect elevated concentrations of Ca\(^{2+}\) to reduce AgNP toxicity due to simultaneous protection at the biotic ligand and the rapid aggregation of AgNPs\(^{12-21}\). Aggregation reduces the surface area of the reactive surface of the solid particle that could dissolve silver ions, and large particles sink rapidly thereby removing AgNPs from the water column where they potentially have the greatest ability to have an effect\(^{31-33}\). Na\(^+\) has been shown to elicit the same aggregation effect as Ca\(^{2+}\) but at higher concentrations. It is likely that the Na\(^+\) concentrations present in my toxicity tests will be too low to produce noticeable aggregation effects, which will highlight the effects caused by the elevated anion concentrations in those tests.

If the BLM is the strongest predictor of AgNP toxicity then mortality of *D. magna* will be strongly correlated with dissolved Ag\(^+\) concentration. However, if toxicity does not follow BLM predictions, this will indicate that there is a separate mechanism of toxicity related specifically to the particulate form and behavior of AgNPs. Exploring these differences in freshwater ion composition will highlight the importance of particle characteristics versus environmental conditions on the toxicity of AgNPs to *Daphnia magna* and, by extension, other organisms that have been proven to exhibit Ag\(^+\) toxicity in accordance with the BLM.

**Timeline of Project:**

<table>
<thead>
<tr>
<th>Year</th>
<th>Activity</th>
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<tbody>
<tr>
<td>May 2017</td>
<td>Preliminary analytical tests: Determining AgNP behavior in each toxicity test condition, refining techniques, etc.</td>
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<tr>
<td>June 2017</td>
<td><em>Daphnia</em> range-finding tests</td>
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<tr>
<td>Early July 2017</td>
<td><em>Daphnia</em> toxicity tests</td>
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<tr>
<td>July – Sept. 2017</td>
<td>AgNP characterization tests</td>
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<td>Total number of daphnids broken down:</td>
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<tr>
<td><strong>Daphnia magna</strong></td>
<td><strong>Toxicity Tests:</strong> 2 replicates of each test, 8 tests, 7 concentrations / test, 3 replicates / concentration, 5 daphnids / replicate</td>
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<tr>
<td></td>
<td><strong>Range Finding Tests:</strong> 8 waters to find ranges for, 8 concentrations per water, 5 daphnids per concentration</td>
</tr>
<tr>
<td></td>
<td><strong>Reference Tests:</strong> (required by ASTM methods for each day of testing) 2 days of testing, 7 concentrations, 3 replicates / concentration, 5 daphnids / replicate</td>
</tr>
</tbody>
</table>

**Total number of daphnids:** 2210

**Cost ($0.25 per each):** $552.50

**Shipping ($60 / shipment * 3 shipments):** $180.00

**Total cost of Daphnia:** $732.50

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<thead>
<tr>
<th></th>
<th>Total hours broken down:</th>
<th>Number of hours to run samples</th>
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<tbody>
<tr>
<td><strong>ICP-MS costs</strong></td>
<td><strong>Single particle Analysis:</strong> (1 sample / hour) 8 waters, 2 replicates / water</td>
<td>16</td>
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<tr>
<td></td>
<td><strong>Single particle calibration curves:</strong> 3 hours of cal curves for each of 8 waters</td>
<td>24</td>
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<tr>
<td></td>
<td><strong>Dissolved supernatant:</strong> (6 samples / hour) 8 waters, 2 replicates / water</td>
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<td><strong>Total hours:</strong></td>
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<td></td>
<td><strong>Cost (ICP-MS = $10 / hour)</strong></td>
<td>$430.00</td>
</tr>
</tbody>
</table>

**Total Project Costs Requested:** $1,162.50

**Other sources of funding:** $0.00
References Cited


