

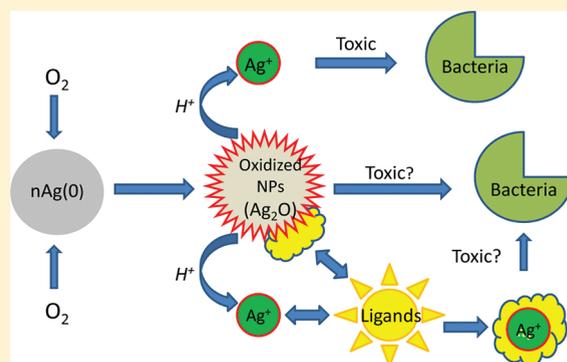
Differential Effect of Common Ligands and Molecular Oxygen on Antimicrobial Activity of Silver Nanoparticles versus Silver Ions

Zong-Ming Xiu, Jie Ma, and Pedro J. J. Alvarez*

Department of Civil & Environmental Engineering, Rice University, Houston, Texas 77005, United States

Supporting Information

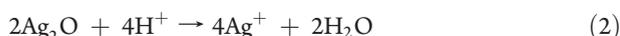
ABSTRACT: The antibacterial activity of silver nanoparticles (AgNPs) is partially due to the release of Ag^+ , although discerning the contribution of AgNPs vs Ag^+ is challenging due to their common co-occurrence. We discerned the toxicity of Ag^+ versus a commercially available AgNP (35.4 ± 5.1 nm, coated with amorphous carbon) by conducting antibacterial assays under anaerobic conditions that preclude Ag(0) oxidation, which is a prerequisite for Ag^+ release. These AgNPs were 20× less toxic to *E. coli* than Ag^+ (EC_{50} : 2.04 ± 0.07 vs 0.10 ± 0.01 mg/L), and their toxicity increased 2.3-fold after exposure to air for 0.5 h (EC_{50} : 0.87 ± 0.03 mg/L) which promoted Ag^+ release. No significant difference in Ag^+ toxicity was observed between anaerobic and aerobic conditions, which rules out oxidative stress by ROS as an important antibacterial mechanism for Ag^+ . The toxicity of Ag^+ (2.94 μmol/L) was eliminated by equivalent cysteine or sulfide; the latter exceeded the solubility product equilibrium constant (K_{sp}), which is conducive to silver precipitation. Equivalent chloride and phosphate concentrations also reduced Ag^+ toxicity without exceeding K_{sp} . Thus, some common ligands can hinder the bioavailability and mitigate the toxicity of Ag^+ at relatively low concentrations that do not induce silver precipitation. Furthermore, low concentrations of chloride (0.1 mg/L) mitigated the toxicity of Ag^+ but not that of AgNPs, suggesting that previous reports of higher AgNPs toxicity than their equivalent Ag^+ concentration might be due to the presence of common ligands that preferentially decrease the bioavailability and toxicity of Ag^+ . Overall, these results show that the presence of O_2 or common ligands can differentially affect the toxicity of AgNPs vs Ag^+ , and underscore the importance of water chemistry in the mode of action of AgNPs.



INTRODUCTION

Silver nanoparticles (AgNPs) are widely used in consumer products such as textiles, personal care, and food storage containers for their potent antibacterial capacity.^{1–12} AgNP-containing products compose the largest group (55.4%) of all of the nanobased consumer products available on the market as of March, 2011.¹³ Thus, the incidental or accidental release of AgNPs to the environment represents a potential risk to a wide variety of organisms, including indigenous microbial communities that provide critical ecosystem services (e.g., primary productivity, nutrient cycling, waste degradation, and climate regulation).¹⁴ However, the mechanisms by which AgNPs exert toxicity are not fully understood, and the role of water chemistry in the mode of action of AgNPs versus released Ag^+ has received limited attention.

Zero-valent silver metal is insoluble in water,¹⁵ but Ag^+ can be released from AgNPs (eq 2) following oxidation of Ag(0) on the nanoparticle surface (eq 1):¹⁶



The released Ag^+ is toxic to bacteria due to various mechanisms

that include binding to thiol groups in proteins and disrupting their function, compromising membrane permeability leading to cell lysis and death,^{17,18} and oxidative stress due to generation of reactive oxygen species (ROS).^{9,19,20} However, discerning the contribution of Ag^+ vs the AgNPs themselves is challenging due to their common co-occurrence during the exposure period, because most antibacterial assays are conducted under aerobic conditions that promote continuous Ag^+ release (Figure 1). Some studies suggest that dissolved Ag^+ accounts for most if not all of AgNPs' toxicity, and AgNPs serve mostly as a source of Ag^+ .^{6,21} These studies used ligands such as cysteine²¹ to neutralize Ag^+ and isolate the effect of AgNPs, but this approach may confound the reactivity of the AgNPs themselves due to their potential association with the added ligand. Other studies showed that both AgNPs and Ag^+ contribute to the antibacterial activity⁷ and toxicity to eukaryotes,^{22–26} although their apparent relative importance varies considerably. For example, the toxicity of AgNPs to bacteria (*E. coli*, *B. subtilis*, and *S. oneidensis*)²⁶ and to

Received: June 5, 2011

Accepted: September 9, 2011

Revised: September 9, 2011

Published: September 27, 2011

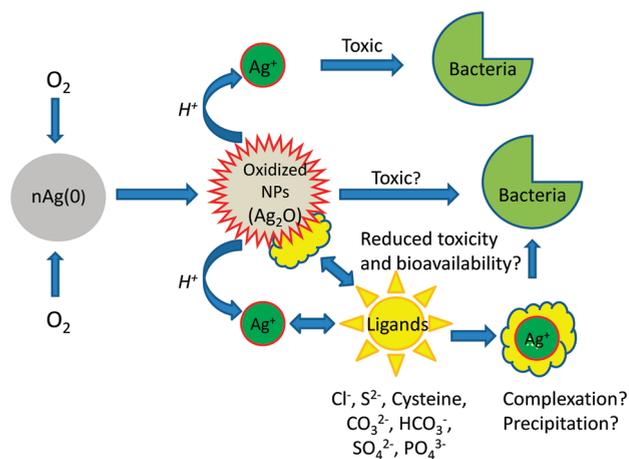


Figure 1. Role of oxygen and common ligands on the antibacterial activity of AgNPs.

the ryegrass *Lolium multiflorum*²³ can be higher than that exerted by an equivalent Ag⁺ concentration, although the mechanism responsible for higher toxicity was not discerned.

The antibacterial activity of AgNPs can depend on particle size,^{5,27–29} shape,¹⁰ and surface charge.¹² However, most commercially available AgNPs are stabilized by various types of organic coatings, and the effect of these coatings on Ag⁺ release and antibacterial activity has not been systematically addressed in the literature. In fact, the concentration of Ag⁺ in most toxicity studies with AgNPs is either missing or not explicitly mentioned.¹² Another potential confounding factor is the presence of common ligands in water (e.g., Cl⁻, PO₄³⁻, S²⁻, and SO₄²⁻) which can associate with Ag⁺ and induce its precipitation, thus reducing Ag⁺ bioavailability and toxicity.^{17,18,30} Furthermore, Ag⁺ and some potential ligands are likely to occur in relatively low concentrations that form soluble complexes rather than precipitates. Thus, it is important to consider how such common ligands affect the ecotoxicity of Ag⁺ (or its disinfection efficacy) when present at concentrations that are below their solubility product equilibrium constant (K_{sp}).

This paper compares the toxicity of Ag⁺ versus a commercially available AgNP suspension by conducting antibacterial assays under both aerobic and anaerobic conditions. The latter eliminated confounding effects associated with oxidative release of Ag⁺. The differential effect of common ligands on AgNP vs Ag⁺ toxicity was also considered to address how water chemistry may affect their relative contribution to antimicrobial activity.

MATERIALS AND METHODS

Chemicals and AgNPs. AgNPs (coated with amorphous carbon), which have been used in previous studies,^{5,31} were obtained from Novacentrix Corporation (Austin, TX). These particles had a mean size of 35.4 ± 5.1 nm in the exposure medium (2 mM sodium bicarbonate buffer), determined by dynamic light scattering with a Malvern Zetasizer (ZEN 3600, Malvern Instrument, U.K.), and a ζ potential (ζ) of -27.0 ± 1.54 mV when prepared and stored anaerobically vs -30.0 ± 0.42 mV after 10-day equilibration with air, as measured by the same instrument. The nano powder was suspended in Milli-Q water and homogenized by an ultrasonic cleaner (5510, Branson, CT).

The metal basis purity of the AgNPs is 99.92%, as measured by ICP-OES (Perkin-Elmer, Waltham, MA). AgNO₃ and HNO₃ (~69.0%) was obtained from Sigma-Aldrich (St. Louis, MO); Na₂S, NaCl, cysteine, LB (Luria–Bertani) broth, NaHCO₃ and H₂O₂ (30%) were all obtained from Fisher Scientific (Fair Lawn, NJ). All chemicals used were reagent grade or better unless otherwise specified.

Bacteria. *E. coli* strain K12 (ATCC 25404) was chosen as a model microorganism for inactivation experiments. A single colony of *E. coli* grown on LB agar plates was inoculated in 10 mL of LB Broth and grown in a shaking incubator at 37 °C overnight. The bacteria were harvested by centrifugation at $10\,000 \times g$ for 1 min, washed three times with sodium bicarbonate buffer (2 mM, pH 8.1), and resuspended in the same sodium bicarbonate buffer (10 mL) to make the bacteria stock solution. Sodium bicarbonate can buffer the system at relatively low ionic strength (which promotes nanoparticle coagulation) compared to other bacteria media, and was chosen as the exposure medium to avoid ligands that could bind with Ag⁺/AgNPs and promote precipitation or other confounding effects. Bacteria (1 mL) were added respectively into test tubes to achieve a viable cell concentration of about 10^7 cells/mL.

Preparation of AgNP Stock Solutions. The commercial AgNPs were washed five times with 1% HNO₃ and then another five times with deoxygenated (N₂-purged) water inside an anaerobic chamber to remove dissolved Ag⁺ and oxidized silver from the AgNPs, prior to filtration through a cellulose membrane (molecular weight cutoff 10 000) using a Amicon stir cell (Millipore, MA) pressurized with nitrogen. The filtrate was analyzed for total dissolved silver with an ICP-OES/MS (Perkin-Elmer, Waltham, MA) to obtain the concentrations of dissolved silver (mainly Ag⁺). The AgNPs were washed and filtered continuously until the Ag⁺ concentration in filtrate was lower than 300 μg/L. This corresponds to a maximum diluted Ag⁺ concentration of 3 μg/L in the exposure medium, which below the minimum lethal concentration (MLC, 25.4 μg/L) of Ag⁺ to *E. coli*. The MLC is defined here as the minimum concentration of Ag⁺ in the exposure medium (bicarbonate buffer) that causes statistically significant *E. coli* mortality ($p < 0.05$) relative to the control set without silver, and was determined by the same procedure described below for the dose–response assays (For details please refer to Supporting Information (SI), Figure S1). For reference, the minimum inhibitory concentration (MIC), which is determined by standard procedures in a different medium (LB broth)³² was measured at 5.25 mg/L for Ag⁺.

After filtration, the retentate was rinsed with Milli-Q water to resuspend the AgNPs, and was then transferred to 50-mL corning tubes (Corning, Lowell, MA) and sonicated at low intensity for 10 s (Sonic Ruptor 250, Omni International, GA) inside the anaerobic chamber to disaggregate AgNPs. This stock was stored and tested inside the anaerobic chamber to avoid confounding effect caused by oxidative Ag⁺ release during the dose–response assays. For the aerobic AgNPs dose–response test, an aliquot of AgNPs were transferred out of the anaerobic chamber and equilibrated with air for 0.5-h or 10-day prior to inoculation.

The concentration of the AgNPs stock was determined by nitric acid/hydrogen peroxide digestion (2 mL HNO₃, 1 mL H₂O₂). AgNPs stock (100 μL) was digested in 20-mL disposable scintillation vials for 24 h in triplicate, followed by filtration through 0.22 μm filter (Fisher Scientific, NJ) to get rid of

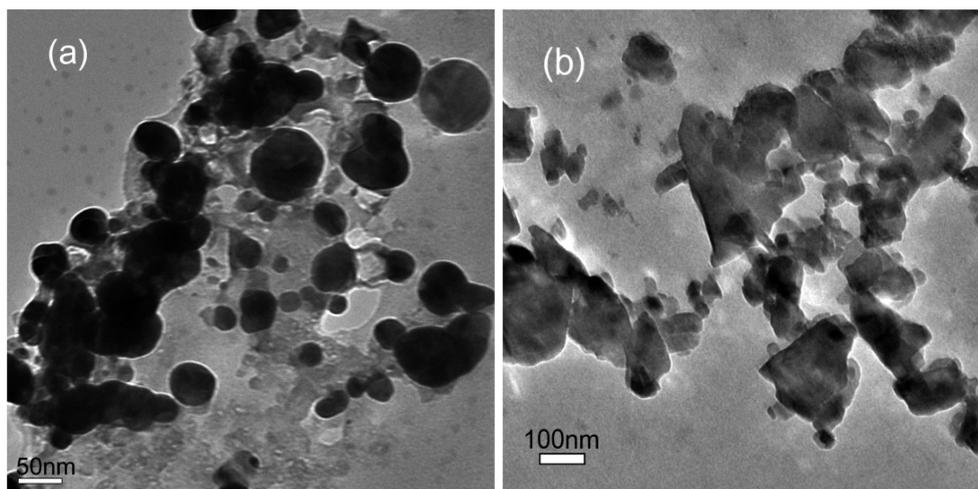


Figure 2. TEM images of AgNPs stored under (a) anaerobic conditions and (b) aerobic conditions after 10-day exposure to air.

impurities. The resulting filtrate volume (~ 3 mL) was brought to 10 mL with 1% nitric acid (100-fold dilution) and its concentration was then determined by ICP-OES (detection limit $80 \mu\text{g/L}$) or ICP/MS (detection limit $1 \mu\text{g/L}$). The Ag^+ concentrations in the stock solution (including treatments exposed to air for 0.5 h and 10 days) were determined by filtering 1 mL stock solution through the cellulose membrane and measuring the total dissolved silver concentration in the filtrate by ICP-MS.

Dose–Response of AgNPs and Ag^+ . The AgNPs stock solution was diluted in 10 mL of sodium bicarbonate buffer (2 mM, pH 8.1) to obtain different concentrations (up to 6.2 mg/L), mixed, and equilibrated for 0.5 h before adding *E. coli* and incubating in the dark for 6 h at 23°C under anaerobic (inside the chamber) or aerobic conditions (outside the chamber). *E. coli* mortality in different treatments was then determined by viable plate counts.³³ Briefly, all solutions were serially diluted and eight $10\text{-}\mu\text{L}$ droplets from each dilution were placed on LB agar plates. The plates were incubated at 37°C for 8 h, and the colony forming units (CFU) were counted. The bacteria mortality was calculated as $1 - N/N_0 \times 100\%$, where N and N_0 are the remaining and initial concentrations of viable bacteria (CFU/mL), respectively. Dose–response assays were prepared similarly for Ag^+ . All tests were conducted in triplicate and repeated at least three times to ensure reproducibility.

For dose–response assays to study the effect of common ligands, equivalent concentrations of each ligand (sulfide, cysteine, chloride, and phosphate) were added separately (0.5 h before addition of bacteria) as sodium salts. Ligands were also tested separately without Ag^+ or AgNPs to ensure that they did not inhibit the bacteria.

Transmission Electron Microscopy (TEM). To compare the different morphology of AgNPs under aerobic versus anaerobic conditions, TEM samples were prepared inside and outside the anaerobic chamber. For the anaerobic sample, a homogeneous diluted suspension ($5 \mu\text{L}$) of the filtered AgNPs sample was deposited on a 400-mesh copper grid (Ultrathin carbon type-A, Ted Pella Inc., Redding, CA) and dried inside the chamber. The aerobic sample was prepared similarly except that it was air-dried outside the chamber. Imaging was performed by TEM using a JEOL 2010 (JEOL, Tokyo, Japan) operated at 120 kV.

Statistical Analyses. Whether differences between treatments were statistically significant was determined using Student's *t* test

at the 95% confidence level. All measurements are reported as mean \pm one standard deviation with three replicates.

RESULTS AND DISCUSSION

TEM Characterization of Aerobically and Anaerobically Prepared AgNPs. TEM analysis showed that anaerobically prepared AgNPs had a spherical shape with a relatively smooth surface (Figure 2a). Following 0.5-h or 10-day exposure to air, the AgNPs had an irregular shape with a rough surface indicative of surface oxidation (Figure 2b). This likely reflects the instability of Ag^0 in the presence of oxygen, which can react to form silver oxide (cubic crystal) on the AgNP surface.^{16,34} The anaerobically prepared AgNPs tended to adhere to the walls of (hydrophobic) polypropylene tubes (SI Figure S2) while the AgNPs that were equilibrated with air for 10 days did not, indicating that the absence of molecular oxygen could affect the hydrophobicity and adsorption characteristics of AgNPs. This corroborates the importance of hydrophobic interactions in the attachment of AgNPs to hydrophobic surfaces³⁵ and suggests their potential significance in both the transport of AgNPs and their affinity for bacterial surfaces.

Ag^+ Toxicity under Aerobic versus Anaerobic Conditions. A comparison of AgNP toxicity under aerobic versus anaerobic conditions requires consideration of potential confounding effects associated with Ag^+ release, which includes determining whether Ag^+ exerts differential toxicity under aerobic versus anaerobic conditions. Figure 3(a) shows that this is not the case; Ag^+ exerted similar toxicity under both aerobic and anaerobic conditions. Specifically, *E. coli* mortality was $97.7 \pm 3.5\%$ following 6-h exposure to $320 \mu\text{g/L}$ Ag^+ under anaerobic conditions, compared to $99.8 \pm 8.9\%$ under aerobic conditions, and the corresponding EC_{50} values were statistically undistinguishable ($p > 0.05$) (93.4 ± 4.4 v. $95.6 \pm 7.5 \mu\text{g/L}$).

One of the most commonly proposed mechanisms for the antibacterial effect of Ag^+ is the generation of ROS,^{9,20} which requires the presence of O_2 as a precursor.^{20,36} Since ROS could not be produced during anaerobic exposure, the lack of significant difference in toxicity under aerobic versus anaerobic conditions indicates that ROS-induced oxidative stress is not the dominant antibacterial mechanism for Ag^+ . Other mechanisms

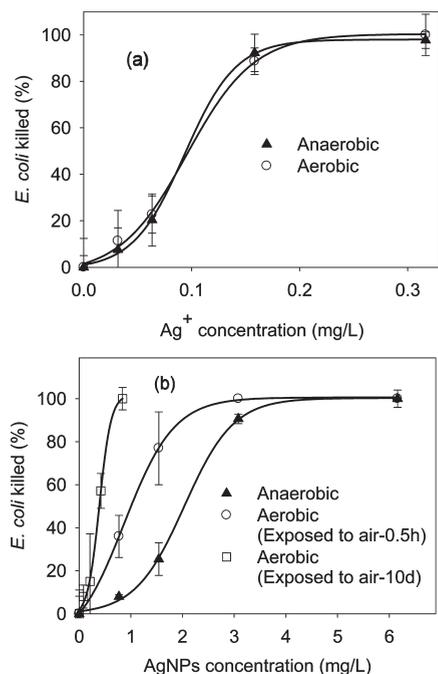


Figure 3. Toxicity of (a) Ag^+ and (b) AgNPs under aerobic vs anaerobic conditions. The AgNPs were prepared under anaerobic conditions and tested inside the chamber or outside after exposure to air for 0.5-h (releasing up to $76 \mu\text{g/L Ag}^+$) or 10-day (releasing up to $181 \mu\text{g/L Ag}^+$).

such as the inactivation of thiol-containing proteins^{17,18} appear to be more important.

Discerning the toxicity of AgNPs versus Ag^+ . In order to quantify the relative contributions of released Ag^+ versus the AgNPs themselves, the nanoparticles were filtered and their dose–response patterns were compared under both aerobic and anaerobic conditions (Figure 3). The maximum dose of the anaerobically prepared AgNPs (6.2 mg/L), which resulted in $97.7 \pm 3.5\%$ mortality, contained a residual Ag^+ concentration ($3.0 \mu\text{g/L}$) that is lower than the MLC for *E. coli* ($25.4 \mu\text{g/L}$). Thus, Ag^+ released to the bulk solution did not contribute to the antibacterial activity of AgNPs in this assay. Albeit, on the basis of EC_{50} values, anaerobically prepared filtered AgNPs (Figure 3b) were about $20\times$ less toxic than Ag^+ (Figure 3a) (EC_{50} : 2.04 ± 0.07 vs 0.10 ± 0.01 mg/L).

The higher bioavailability and uptake potential of Ag^+ compared to AgNPs might explain its higher toxicity. The negatively charged bacteria surface ($\zeta = -26.9 \pm 2.6$ mV) should have higher affinity for Ag^+ than the negatively charged AgNPs ($\zeta = -30.0 \pm 0.4$ for aerobic suspension). AgNPs might be more difficult to be assimilated by bacterial cells due to their relatively large size (35.4 ± 5.1 nm) compared to Ag^+ (0.26 nm).³⁷ The cell membrane and cytoplasm contain many sulfur-containing proteins that Ag^+ can bind to and inactivate.³⁸ Ag^+ may also bind to phosphate-containing molecules such as DNA to harm cells. Although small AgNPs (1–10 nm) can penetrate the cell membrane of *E. coli*,⁵ the uptake of larger nanoparticles by bacteria has been shown to be more difficult.³⁹ If AgNPs get into the cytoplasm, then they could theoretically inactivate proteins and DNA, or be oxidized gradually by intracellular ROS, resulting in the release of Ag^+ to eventually increase the intracellular toxicity of AgNPs.²³

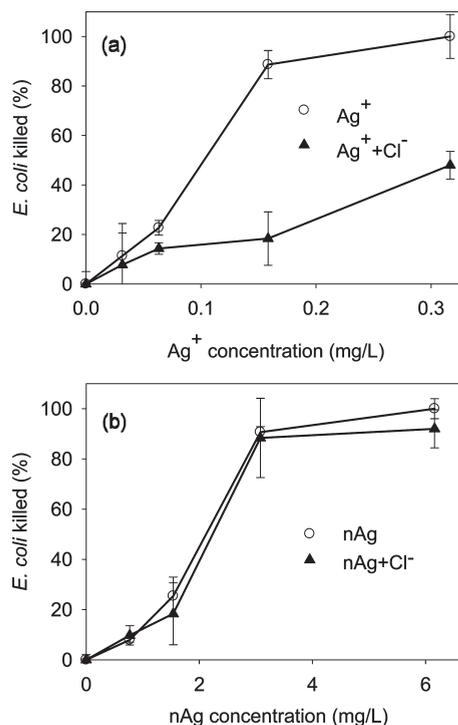


Figure 4. Chloride (at equivalent concentrations to the highest silver dose tested) significantly mitigated the toxicity of Ag^+ (a), but not AgNPs (b). Exposure to Ag^+ was under aerobic conditions with $2.94 \mu\text{mol/L}$ chloride, whereas exposure to AgNPs was under anaerobic conditions with $57.0 \mu\text{mol/L}$ chloride.

To assess how the oxidation of AgNPs influences their toxicity, dose–response assays were also conducted with the same anaerobically filtered AgNPs, except that exposure to bacteria was conducted outside the anaerobic chamber after 0.5-h or 10-day equilibration with air. Antibacterial activity increased with AgNPs exposure to air. On the basis of EC_{50} values, a $2.3\times$ higher toxicity was observed after 0.5-h exposure to air (EC_{50} : 0.87 ± 0.03 mg/L) and $5.1\times$ higher toxicity after 10-day aeration (EC_{50} : 0.87 ± 0.03 mg/L) relative to anaerobic exposure (EC_{50} : 2.04 ± 0.07 mg/L) (Figure 3b), even though aerobic conditions increased the negative charge of AgNPs (ζ increased from -27.0 ± 1.5 mV when stored anaerobically to -30.0 ± 0.4 mV after 10-day exposure to air), which is conducive to higher electrostatic repulsion with the negatively charged *E. coli* cells ($\zeta = -26.9 \pm 2.6$ mV). Apparently, the presence of molecular oxygen promoted the release of Ag^+ ^{16,34} (the Ag^+ concentration for the maximum AgNPs dose of 6.2 mg/L was $76 \mu\text{g/L}$ for the treatment exposed to air for 0.5-h, and $181 \mu\text{g/L}$ for the treatment exposed to air for 10-days) and increased AgNP toxicity. This underscores the importance to discern the rate and extent of oxidative Ag^+ release in studies of the potential eco-toxicity or disinfection capacity of AgNPs.

Common Ligands Mitigate Toxicity of AgNPs and Ag^+ Differentially. Common ligands that are prevalent in aquatic systems (e.g., Cl^- , S^{2-} , cysteine, phosphate) may mitigate the toxicity of Ag^+ and AgNPs differentially, thus affecting their relative contribution to antibacterial activity. Figure 4 shows that low equimolar concentrations of chloride (Ag^+ and Cl^- at $2.94 \mu\text{mol/L}$ (0.1 mg/L)) reduced the toxicity of Ag^+ by about 55%, while the toxicity of AgNPs decreased by only 9% in the presence

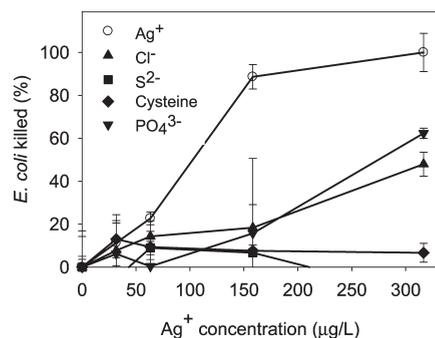


Figure 5. Equivalent ligand concentrations mitigated the toxicity of Ag⁺ (2.94 µmol/L) by promoting silver precipitation (with sulfide: 1.47 µmol/L or cysteine: 2.94 µmol/L) or complexation (with chloride: 2.94 µmol/L or phosphate: 0.98 µmol/L) under aerobic conditions.

of higher chloride concentration (AgNPs and Cl⁻ at 57.4 µmol/L (2.0 mg/L)). These chloride concentrations are much lower than those commonly found in fresh water.⁴⁰ The preferential mitigation of Ag⁺ over AgNPs toxicity by a common ligand may partially explain previous reports of higher AgNPs toxicity than their equivalent Ag⁺ concentrations.^{23,26} In theory, AgNPs could become more effective means to deliver Ag⁺ to cells when the bioavailability of the ions is hindered by complexation and/or precipitation with common ligands. Thus, the lower susceptibility of AgNPs may be due to their ability to more effectively deliver Ag⁺ to the bacteria after attachment to the cell surface or intracellular uptake.⁵

Several other ligands (e.g., S²⁻, cysteine, phosphate) were also added separately to compare their potential to reduce the toxicity of Ag⁺ (Figure 5). The toxicity of Ag⁺ was completely removed by the addition of cysteine and sulfide ($K_{sp-Ag_2S} = 1 \times 10^{-50.1}$), which have low solubility products that are conducive to silver precipitation (SI Table S1). Significant toxicity reduction was also observed by chloride (55% at the lethal dose) and phosphate (50%). Unlike sulfide and cysteine, chloride and phosphate have relatively high solubility product equilibrium constants ($K_{sp-AgCl} = 1 \times 10^{-9.7}$ and $K_{sp-Ag_3PO_4} = 1 \times 10^{-17.6}$) that were not exceeded by our tested concentrations (SI Table S1). Therefore, some natural ligands can mitigate the toxicity of Ag⁺ by forming aqueous complexes below the precipitation potential.

Overall, the tested commercial AgNPs (35.4 ± 5.1 nm) were significantly less toxic to *E. coli* than Ag⁺, although the presence of common ligands such as chloride, phosphate, and sulfide could alter their relative contribution to antibacterial activity by complexing with Ag⁺ and preferentially decreasing its toxicity. Although the exact toxicity mechanism(s) for AgNPs was not elucidated, the similar toxicity of Ag⁺ under aerobic and anaerobic conditions rules out ROS generation (which requires the presence of O₂) as a major toxicity mechanism. The mitigation of Ag⁺ toxicity by low concentrations of common ligands (even at levels that do not induce silver precipitation) suggest a potentially significant natural mechanism to attenuate toxicity, and underscores the importance to consider water chemistry in efforts to use silver as a disinfectant or elucidate the antibacterial mechanisms of AgNPs.

■ ASSOCIATED CONTENT

Supporting Information. Details on the determination of the minimum lethal concentration (MLC), adherence of

AgNPs to polypropylene tubes, and a comparison of Ag⁺ and ligands concentration with the corresponding solubility product equilibrium constant (K_{sp}). This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (713) 348-5903, e-mail: alvarez@rice.edu.

■ ACKNOWLEDGMENT

This research was sponsored by a Joint U.S.–U.K. Research Program (U.S.-EPA and U.K.-NERC-ESPRC) (EPA -G2008-STAR-R1). We thank Gautam Kini (Rice University) for his assistance with the particle size and zeta-potential measurements.

■ REFERENCES

- (1) Shahverdi, A. R.; Fakhimi, A.; Shahverdi, H. R.; Minaian, S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. *Nanomed.-Nanotechnol.* **2007**, *3* (2), 168–171.
- (2) Shrivastava, S.; Bera, T.; Roy, A.; Singh, G.; Ramachandrarao, P.; Dash, D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology* **2007**, *18*, (22), 225103.
- (3) Yoon, K. Y.; Byeon, J. H.; Park, J. H.; Hwang, J. Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles. *Sci. Total Environ.* **2007**, *373* (2–3), 572–575.
- (4) Sondi, I.; Salopek-Sondi, B. Silver nanoparticles as antimicrobial agent: A case study on *E-coli* as a model for Gram-negative bacteria. *J. Colloid Interface Sci.* **2004**, *275* (1), 177–182.
- (5) Morones, J. R.; Elechiguerra, J. L.; Camacho, A.; Holt, K.; Kouri, J. B.; Ramirez, J. T.; Yacaman, M. J. The bactericidal effect of silver nanoparticles. *Nanotechnology* **2005**, *16* (10), 2346–2353.
- (6) Lok, C. N.; Ho, C. M.; Chen, R.; He, Q. Y.; Yu, W. Y.; Sun, H.; Tam, P. K. H.; Chiu, J. F.; Che, C. M. Silver nanoparticles: Partial oxidation and antibacterial activities. *J. Biol. Inorg. Chem.* **2007**, *12* (4), 527–534.
- (7) Fabrega, J.; Fawcett, S. R.; Renshaw, J. C.; Lead, J. R. Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. *Environ. Sci. Technol.* **2009**, *43* (19), 7285–7290.
- (8) Choi, O.; Deng, K. K.; Kim, N. J.; Ross, L.; Surampalli, R. Y.; Hu, Z. Q. The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Res.* **2008**, *42* (12), 3066–3074.
- (9) Choi, O.; Hu, Z. Q. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. *Environ. Sci. Technol.* **2008**, *42* (12), 4583–4588.
- (10) Pal, S.; Tak, Y. K.; Song, J. M. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. *Appl. Environ. Microb.* **2007**, *73* (6), 1712–1720.
- (11) Kim, J. S.; Kuk, E.; Yu, K. N.; Kim, J. H.; Park, S. J.; Lee, H. J.; Kim, S. H.; Park, Y. K.; Park, Y. H.; Hwang, C. Y.; Kim, Y. K.; Lee, Y. S.; Jeong, D. H.; Cho, M. H. Antimicrobial effects of silver nanoparticles. *Nanomed.-Nanotechnol.* **2007**, *3* (1), 95–101.
- (12) El Badawy, A. M.; Silva, R. G.; Morris, B.; Scheckel, K. G.; Suidan, M. T.; Tolaymat, T. M. Surface charge-dependent toxicity of silver nanoparticles. *Environ. Sci. Technol.* **2011**, *45* (1), 283–7.
- (13) Rejeski, D.; Kulken, T.; Pollschuck, P.; Pauwels, E. *The project on emerging nanotechnologies*. http://www.nanotechproject.org/inventories/consumer/analysis_draft/, 2011.
- (14) Munn, C. *Marine Microbiology*; Oxford: BIOS Scientific, 2003.
- (15) Petrucci, R. H.; Harwood, W. S.; Herring, G. E.; Madura, J. *General Chemistry: Principles and Modern Applications*, 9th ed.; Prentice Hall: New York, 1997.

- (16) Liu, J. Y.; Hurt, R. H. Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environ. Sci. Technol.* **2010**, *44* (6), 2169–2175.
- (17) Ratte, H. T. Bioaccumulation and toxicity of silver compounds: A review. *Environ. Toxicol. Chem.* **1999**, *18* (1), 89–108.
- (18) Wang, J. M.; Huang, C. P.; Pirestani, D. Interactions of silver with wastewater constituents. *Water Res.* **2003**, *37* (18), 4444–4452.
- (19) Kim, S.; Choi, J. E.; Choi, J.; Chung, K. H.; Park, K.; Yi, J.; Ryu, D. Y. Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. *Toxicol. in Vitro* **2009**, *23* (6), 1076–1084.
- (20) Park, H. J.; Kim, J. Y.; Kim, J.; Lee, J. H.; Hahn, J. S.; Gu, M. B.; Yoon, J. Silver-ion-mediated reactive oxygen species generation affecting bactericidal activity. *Water Res.* **2009**, *43* (4), 1027–1032.
- (21) Navarro, E.; Piccapietra, F.; Wagner, B.; Marconi, F.; Kaegi, R.; Odzak, N.; Sigg, L.; Behra, R. Toxicity of Silver Nanoparticles to *Chlamydomonas reinhardtii*. *Environ. Sci. Technol.* **2008**, *42* (23), 8959–8964.
- (22) Meyer, J. N.; Lord, C. A.; Yang, X. Y. Y.; Turner, E. A.; Badireddy, A. R.; Marinakos, S. M.; Chilkoti, A.; Wiesner, M. R.; Auffan, M. Intracellular uptake and associated toxicity of silver nanoparticles in *Caenorhabditis elegans*. *Aquat. Toxicol.* **2010**, *100* (2), 140–150.
- (23) Yin, L. Y.; Cheng, Y. W.; Espinasse, B.; Colman, B. P.; Auffan, M.; Wiesner, M.; Rose, J.; Liu, J.; Bernhardt, E. S. More than the ions: the effects of silver nanoparticles on *Lolium multiflorum*. *Environ. Sci. Technol.* **2011**, *45* (6), 2360–2367.
- (24) Kawata, K.; Osawa, M.; Okabe, S. In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells. *Environ. Sci. Technol.* **2009**, *43* (15), 6046–6051.
- (25) Laban, G.; Nies, L. F.; Turco, R. F.; Bickham, J. W.; Sepulveda, M. S. The effects of silver nanoparticles on fathead minnow (*Pimephales promelas*) embryos. *Ecotoxicology* **2010**, *19* (1), 185–195.
- (26) Suresh, A. K.; Pelletier, D. A.; Wang, W.; Moon, J. W.; Gu, B. H.; Mortensen, N. P.; Allison, D. P.; Joy, D. C.; Phelps, T. J.; Doktycz, M. J. Silver nanocrystallites: biofabrication using *Shewanella oneidensis*, and an evaluation of their comparative toxicity on gram-negative and gram-positive bacteria. *Environ. Sci. Technol.* **2010**, *44* (13), 5210–5215.
- (27) Sotiriou, G. A.; Pratsinis, S. E. Antibacterial activity of nano-silver ions and particles. *Environ. Sci. Technol.* **2010**, *44* (14), 5649–5654.
- (28) Panacek, A.; Kvitek, L.; Prucek, R.; Kolar, M.; Vecerova, R.; Pizurova, N.; Sharma, V. K.; Nevecna, T.; Zboril, R. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. *J. Phys. Chem. B* **2006**, *110* (33), 16248–16253.
- (29) Carlson, C.; Hussain, S. M.; Schrand, A. M.; Braydich-Stolle, L. K.; Hess, K. L.; Jones, R. L.; Schlager, J. J. Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. *J. Phys. Chem. B* **2008**, *112* (43), 13608–13619.
- (30) Choi, O.; Cleuenger, T. E.; Deng, B. L.; Surampalli, R. Y.; Ross, L.; Hu, Z. Q. Role of sulfide and ligand strength in controlling nanosilver toxicity. *Water Res.* **2009**, *43* (7), 1879–1886.
- (31) Zdrov, K.; Brunet, L.; Mahendra, S.; Li, D.; Zhang, A.; Li, Q. L.; Alvarez, P. J. J. Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal. *Water Res.* **2009**, *43* (3), 715–723.
- (32) Andrews, J. M. Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemoth.* **2001**, *48*, 5–16.
- (33) Adams, L. K.; Lyon, D. Y.; Alvarez, P. J. J. Comparative ecotoxicity of nanoscale TiO₂, SiO₂, and ZnO water suspensions. *Water Res.* **2006**, *40* (19), 3527–3532.
- (34) Liu, J. Y.; Sonshine, D. A.; Shervani, S.; Hurt, R. H. Controlled release of biologically active silver from nanosilver surfaces. *ACS Nano* **2010**, *4* (11), 6903–6913.
- (35) Song, J. E.; Phenrat, T.; Marinakos, S.; Xiao, Y.; Liu, J.; Wiesner, M. R.; Tilton, R. D.; Lowry, G. V. Hydrophobic interactions increase attachment of gum arabic- and PVP-coated Ag nanoparticles to hydrophobic surfaces. *Environ. Sci. Technol.* **2011**, *45* (14), 5988–5995.
- (36) Lyon, D. Y.; Brunet, L.; Hinkal, G. W.; Wiesner, M. R.; Alvarez, P. J. J. Antibacterial activity of fullerene water suspensions (*nC(60)*) is not due to ROS-mediated damage. *Nano Lett.* **2008**, *8* (5), 1539–1543.
- (37) Kulinski, K., Environmental impacts of nanosilver: an ICON background. In *ICON: International Council on Nanotechnology*: **2008**.
- (38) Feng, Q. L.; Wu, J.; Chen, G. Q.; Cui, F. Z.; Kim, T. N.; Kim, J. O. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J. Biomed. Mater. Res.* **2000**, *52* (4), 662–668.
- (39) Kloepper, J. A.; Mielke, R. E.; Nadeau, J. L. Uptake of CdSe and CdSe/ZnS quantum dots into bacteria via purine-dependent mechanisms. *Appl. Environ. Microb.* **2005**, *71* (5), 2548–2557.
- (40) Goldman, C. R.; Horne, A. J., *Limnology*; McGraw-Hill Inc.: New York, 1983.