

Environmental Transformations of Silver Nanoparticles: Impact on Stability and Toxicity

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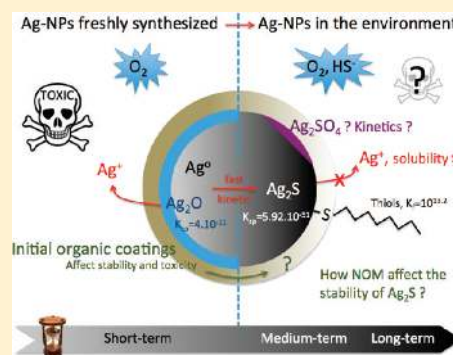
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ABSTRACT: Silver nanoparticles (Ag-NPs) readily transform in the environment, which modifies their properties and alters their transport, fate, and toxicity. It is essential to consider such transformations when assessing the potential environmental impact of Ag-NPs. This review discusses the major transformation processes of Ag-NPs in various aqueous environments, particularly transformations of the metallic Ag cores caused by reactions with (in)organic ligands, and the effects of such transformations on physical and chemical stability and toxicity. Thermodynamic arguments are used to predict what forms of oxidized silver will predominate in various environmental scenarios. Silver binds strongly to sulfur (both organic and inorganic) in natural systems (fresh and sea waters) as well as in wastewater treatment plants, where most Ag-NPs are expected to be concentrated and then released. Sulfidation of Ag-NPs results in a significant decrease in their toxicity due to the lower solubility of silver sulfide, potentially limiting their short-term environmental impact. This review also discusses some of the major unanswered questions about Ag-NPs, which, when answered, will improve predictions about their potential environmental impacts. Research needed to address these questions includes fundamental molecular-level studies of Ag-NPs and their transformation products, particularly Ag₂S-NPs, in simplified model systems containing common (in)organic ligands, as well as under more realistic environmental conditions using microcosm/mesocosm-type experiments. Toxicology studies of Ag-NP transformation products, including different states of aggregation and sulfidation, are also required. In addition, there is the need to characterize the surface structures, compositions, and morphologies of Ag-NPs and Ag₂S-NPs to the extent possible because they control properties such as solubility and reactivity.



1. INTRODUCTION

Silver nanoparticles (Ag-NPs) have recently been the focus of intense research because of the potential risk they pose to humans and other biological organisms.^{1–9} Indeed, the toxicity of silver nanoparticles to a variety of organisms has been demonstrated in a number of recent studies. For example, toxicity has been observed for aquatic (*Lemna minor*)^{10,11} and terrestrial (*Lolium multiflorum*)¹² plants, algae, and fungi,⁴ vertebrates (zebra fish),¹³ invertebrates (*Caenorhabditis elegans*),^{14,15} microorganisms (*Escherichia coli*,^{16,17} *Pseudomonas putida*)¹⁸, and human cells (skin keratinocytes, lung fibroblast cells, and glioblastoma cells).^{19,20} The list of studies showing the negative impact of Ag-NPs on the environment and potentially on humans is long and has been reviewed many times over the past decade.^{1–9} Although the toxicity of Ag-NPs is partly explained by the release of Ag ions, it remains unclear if Ag-NPs are a direct cause of enhanced toxicity. For example, Navarro et al.¹⁰ presented evidence that toxicity is mainly the result of Ag ions and that Ag-NPs contribute to toxicity as a

source of dissolved Ag ions. In contrast, Fabrega et al.²¹ showed a specific nanoparticle effect that could not be explained by dissolved Ag⁺. Similarly, Yin et al.¹² demonstrated that gum arabic-stabilized Ag-NPs more strongly affected the growth of *Lolium multiflorum*, a common grass, more than the equivalent dose of Ag ions added as AgNO₃. They concluded that growth inhibition and cell damage can be directly attributed either to the nanoparticles themselves or to the ability of Ag-NPs to deliver dissolved Ag⁺ to critical biotic receptors. Recently Sotiriou et al.²² proposed that the antibacterial activity of Ag-NPs depends on their size. They provide some evidence that when Ag-NPs are small and release many Ag ions, the

Special Issue: Transformations of Nanoparticles in the Environment

Received: October 21, 2011

Revised: February 1, 2012

Accepted: February 16, 2012

Published: February 16, 2012

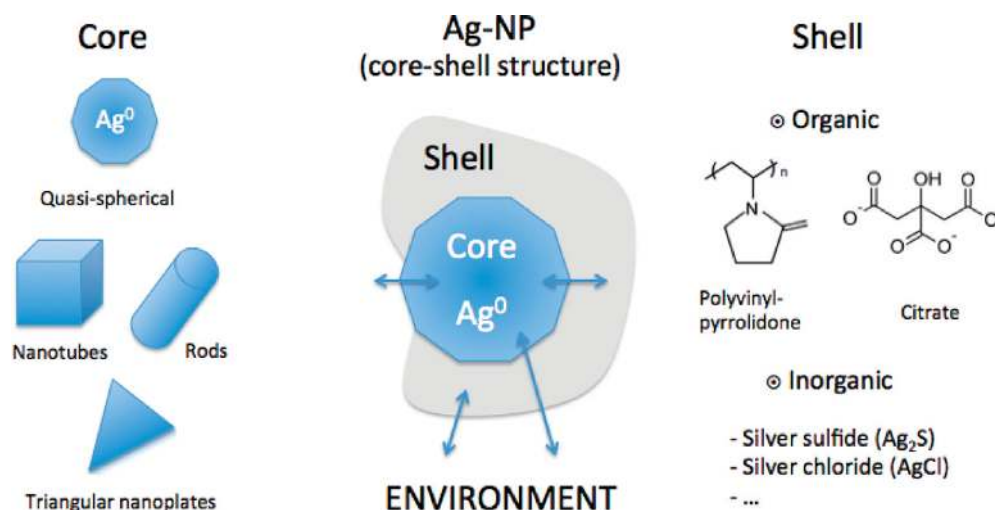


Figure 1. Typical core-shell structure of a Ag-NP that might be released in the environment. Double arrows represent the reactions that might occur between the shell or the core with the environment and also at the interface between core and shell. Note that the shell is usually not a continuous impermeable layer but rather is a discontinuous layer allowing for interaction between the core and the surrounding environment. The discontinuity arises from steric and electrosteric forces between the macromolecules attached to the surface.

antibacterial activity is dominated by these ions. However, when relatively large (mean diameter >10 nm) Ag-NPs are employed, the concentration of released Ag^+ is lower, and the particles themselves also influence Ag-NP antibacterial activity.

Two mechanisms for the toxicity of Ag-NPs to bacteria have been proposed: (1) oxidative stress generated by the formation of reactive oxygen species (ROS) such as oxygen superoxide (O_2^-) that can potentially be formed at the surface of the Ag-NPs^{23–26} and (2) interaction of Ag ions with thiol groups of vital enzymes and proteins, affecting cellular respiration and transport of ions across membranes, with ultimate cell death.^{1,27} More detailed reviews of toxicity and toxicity mechanisms of Ag-NPs to different organisms can be found elsewhere.^{1–9,27} Regardless of toxicity mechanism, increasing production of commercial Ag-NPs will lead to a larger environmental load of Ag species and potential negative impacts on ecosystems. Therefore, the risk of Ag-NPs for humans and the environment needs to be assessed.

Because of the susceptibility of Ag-NPs to environmental transformations (changes in aggregation state, oxidation state, precipitation of secondary phases, sorption of (in)organic species), it is important to assess the toxicity of the transformed nanoparticles as well as the “fresh” ones. It is well-known that metallic Ag is not thermodynamically stable under most environmental conditions and will oxidize or react with (in)organic ligands.^{28,29} For example, silver is known to react strongly with sulfide,³⁰ chloride,³¹ and organic matter.³² Because of their small particle size, the kinetics of corrosion of Ag-NPs are expected to be faster than for bulk silver, reducing greatly the lifetime of the metallic state of Ag in nature. Known silver corrosion agents are ubiquitous in nature, and therefore environmental transformations of Ag-NPs will strongly affect their surface properties²⁹ and consequently their transport, reactivity, and toxicity in soils and aqueous systems. This review discusses the most important environmental transformations of Ag-NPs released into wastewater streams and ecosystems that may affect their stability and toxicity.

Most of the engineered Ag-NPs have a core-shell structure with a Ag^0 core of varying size and shape (e.g., nanospheres, nanocubes, rods, and triangular nanoplates)^{33–35} and an

organic coating with varying molecular weight and functional groups (Figure 1). Engineered Ag-NPs are typically stabilized against aggregation through adsorption or covalent attachment of organic compounds prior to evaluating their environmental fate and toxic effects. These molecules on the surface of the nanoparticles provide electrostatic, steric, or electrosteric repulsive forces between particles, allowing them to resist aggregation.^{36,37} These molecules, if applied during synthesis, are also often referred to as “capping agents” and allow for control of size and shape.³³ In some cases the capping agent (coating) also acts as a reagent to reduce Ag ions to Ag^0 (e.g., citrate). Although the organic layer controls the surface properties of the Ag-NPs, its physical-chemical properties such as purity, surface density, and conformation are rarely if ever measured by manufacturers or researchers because spatially resolving and detecting this organic surface coating is analytically challenging. A 2010 review found that there are many different coatings used to stabilize Ag-NPs against aggregation including carboxylic acids, polymers, polysaccharides, and surfactants.³⁸ Citrate is the most ubiquitous carboxylic acid used as a capping (and reducing) agent,^{38–42} but carboxylic acids with alkyl chains (e.g., oleic acid) are also used.⁴³ Various types of polymers have been used as coatings including polyvinylpyrrolidone,^{30,31,38,39,42,44–46} polyacrylate,⁴⁴ poly(vinylalcohol),^{25,47} polyacrylamide,⁴⁸ and thiol-modified oligonucleotides.⁴⁹ Polysaccharides are common coatings including compounds like gum arabic (GA),^{12,45} sophorolipids,⁵⁰ and other sugars.³⁸ Biological macromolecules such as bovine serum albumin (BSA)¹³ and fatty acids³⁸ have also been used to stabilize Ag-NPs against aggregation. AgNPs can also be embedded in a solid matrix. For example, some work has been done on AgNPs embedded or supported in silica matrices in order to test interesting optical properties.^{22,51,52} In addition, inorganic coatings such as silver carbonate have been used to stabilize Ag-NPs³³ and can form after reaction with inorganic ligands in the environment. Such hermetic coatings may significantly affect AgNP mobility and solubility compared to organic-capped nanoparticles. These coatings define the surfaces of Ag-NPs, in part, and will affect their behavior and transformations in the environment. The molecular weight and

chemical structure of the various organic coatings are very different and therefore will affect adsorbed mass and the conformation of the adsorbed coating, ultimately affecting how well they stabilize the Ag-NPs against aggregation and their behavior in the environment.

Our review begins with a discussion of the surface properties of Ag-NPs coated by organic molecules. The pristine organic shell can be degraded or substituted by natural organic matter (NOM) once released into natural systems. Depending on environmental conditions, these coatings strongly affect Ag-NP surface charge, aggregation, and toxicity. In addition we discuss transformations of the metallic core after exposure to (in)organic compounds for different environmental scenarios such as natural waters and wastewater treatment plants (WWTPs) as most Ag-NPs released from domestic and industrial sources will enter sewer systems.^{54,55} Phase transformations significantly affect the stability of Ag-NPs and consequently their bioavailability and toxicity. The main transformation processes are reviewed in detail as well as the consequences of such transformations on toxicity. Finally, we discuss the current state of knowledge of the behavior of Ag-NPs in the environment as well as pose important unanswered questions that when answered will lead to better understanding of the potential risk of Ag-NPs to the environment.

2. SURFACE PROPERTIES OF AG-NPS: IMPACT ON AGGREGATION AND TOXICITY

2.1. Aggregation State of Ag-NPs. The aggregation state of Ag-NPs is important to evaluate because it impacts the transport and toxicity of these nanomaterials. In general aggregation is an important toxicity-determining parameter for other types of nanomaterials including carbon nanotubes⁵⁶ and metal oxides.⁵⁷ In the case of Ag-NPs, Kvitek et al.⁵⁸ established a correlation between stability against aggregation and the minimum concentration that inhibits the growth of a variety of reference and isolated strains of bacteria. Other studies have demonstrated a clear link between nanoparticle aggregation and loss of an inhibitory effect on bacterial growth.⁵⁹ Moreover, studies of the microbial ecology of estuarine sediments have led to the hypothesis that aggregation mitigates the potential toxicity of Ag-NPs and results in “no effect” to the microbes.⁶⁰

Underlying the lack of complete understanding in this area is the difficulty of precisely measuring environmentally induced changes in NP aggregation state. While a variety of methods for estimating size exist (e.g., dynamic light scattering, DLS; transmission electron microscopy, TEM; atomic force microscopy, AFM; flow field flow fractionation, FFFF; fluorescence correlation spectroscopy, FCS; nanoparticle tracking analysis, NTA), they all have inherent problems and a variety of techniques must be used to minimize errors.⁶¹ The problems become more complex when toxicological assays are performed in a variety of different media types containing various combinations of ions.

Ag-NPs not coated by organic ligands are electrostatically stabilized against aggregation because of their negative surface charge. The charge arises from the presence of negatively charged hydroxo-, oxo-, or, in reducing environments, sulfide groups on the particle surface. Nanoparticles brought into close proximity via Brownian diffusion processes will repel each other if this negative charge is strong enough to overcome aggregation attractive forces (i.e., van der Waals). Electrostatically stabilized Ag-NPs can have their repulsive forces reduced

by the presence of counterions in solution. For example, if these particles are kept in deionized water for weeks prior to experiments, no change in aggregate size or stability is observed.⁶² In contrast, Li et al.⁶³ measured the aggregation of bare Ag-NPs in water over a range of different ionic strengths for three different electrolyte compositions and found that critical coagulation concentrations were >10 mM for monovalent ions and >1 mM for divalent ions. Other research groups have confirmed similar behavior of bare Ag-NPs with a variety of electrolytes.^{64,65}

The more potent aggregation effect of divalent ions (e.g., Ca^{2+}) is well-known in colloid science and has been observed for other nanoparticle systems such as fullerenes.⁶⁶ In addition, the presence of Cl^- seems to promote bridges between nanoparticles, as revealed by TEM,⁶³ that are most likely AgCl precipitates.⁶⁷ The ionic strength of aqueous environmental systems can range from ~1 to 10 mM for freshwater and is ~700 mM for seawater. Because most of these systems contain divalent ions, Ag-NPs without coatings should be unstable in many environments.

Other researchers have modified Ag-NPs with citrate, a small molecular weight chelating agent that stabilizes the particles by increasing the magnitude of the negative surface charge with hydroxo- or oxo- functional groups. These studies have also shown that a significant presence of ions in the environment will effectively destabilize the Ag-NPs, leading to aggregation.^{39,68}

There is a large difference in nanoparticle stability when they are sterically stabilized in addition to being stabilized solely by surface charge. Steric stabilization arises from the presence of polymers attached the surface of NPs often as a “brush-like” layer. When this extended layer approaches another surface the translational and rotational degrees of freedom are reduced, causing a thermodynamically unfavorable loss in entropy. The polymer layer, consequently, resists deposition sterically.³⁷ For example, Ag-NPs have been demonstrated to be disaggregated by Suwannee River humic acid at 10 mg/L^{21,69} and in waters from both a bog lake⁶⁵ and a pond.⁶⁸ Adsorption of compounds found in these natural water samples induces additional steric forces that resist an aggregation process that would otherwise occur. Similarly, the proteins bovine serum albumin and fetal bovine serum have also been shown to stabilize some Ag-NPs.^{59,64}

Steric stabilization due to compound adsorption may be limited when the molecular weights of compounds are smaller than the proteins mentioned above. For example, a fulvic acid concentration of 4.5 mg/L showed little effect on Ag-NP aggregation;⁶³ by definition, fulvic acids have a lower average molecular weight than humic acids. This finding is supported by studies seeking the most suitable stabilizing agents for Ag-NP applications. Kvitek et al.⁵⁸ determined that the high molecular weight polyvinylpyrrolidone (PVP) (360,000) proved to be the most effective polymer for stabilizing Ag-NPs. In addition to steric stabilization polymers with charged groups (i.e., polyelectrolytes) exhibit additional electrosteric forces. Comparing particles stabilized solely by charge (e.g., uncoated H_2O -Ag-NPs and citrate-coated Ag-NPs) with those stabilized by steric forces (PVP-coated Ag-NPs) and electrosteric forces (using branched polyethylenimine (BPEI)-coated Ag-NPs)⁶⁷ revealed a more significant effect on nanoparticles stabilized by electrosteric repulsion. Therefore, as discussed in the next section, polyelectrolytes are often used to stabilize nanoparticle suspensions.

The ability of a polymer to stabilize Ag-NPs depends also on the adsorbed mass and conformation of the adsorbed layer³⁶ and the molecular weight distribution of the polymer.⁷⁰ Some macromolecule types (e.g., PVP³⁹) stabilize Ag-NPs better than others, and this is likely related to their strength of interaction with the Ag-NP surface. However, these interactions need to be explored further to gain a complete understanding of their impact on Ag-NP aggregation.

2.2. Ag-NP Surface Charge and Coating Type Effects on Bacterial Growth Inhibition. The variety of coating types used to stabilize nanoparticles is daunting, but trends revealing the importance of surface charge, hydrophobic groups, and the presence of natural organic matter (NOM) are emerging. Bare Ag-NPs have a negative surface charge throughout the pH range common in the environment (3 to 9);⁶⁷ however, this surface charge can be modified from approximately +30 mV to −60 mV using organic coatings.^{71,72} This variable surface charge may play a large role in bacterial growth inhibition. For example, using the same set of Ag-NPs mentioned previously (bare Ag-NPs, citrate-coated Ag-NPs, PVP-coated Ag-NPs, and BPEI-coated Ag-NPs), El Badawy et al. found that surface charge affected the toxicity of Ag-NPs on *Bacillus* species Gram-positive bacteria.⁶² The three types of negatively charged Ag-NPs exhibited a decrease in magnitude of the measured zeta potential, and this correlated well with increased bacterial growth inhibition (Figure 2).⁶² The positively charged BPEI-coated Ag-NPs were found to be the most effective inhibitors of Gram-positive bacterial growth. They hypothesized that attachment of Ag-NPs to the negatively charged bacterial cell walls is the cause of this charge-dependent toxic effect. Other types of NPs such as gold nanorods have demonstrated a similar toxicity effect due to positively charged coatings.⁷³

Although positive nanoparticle surface charge has a clear inhibitory effect on bacterial growth in some systems, in other cases there is no clear effect caused by altering the surface charge from positive to negative. For example, Dror-Ehre et al.¹⁷ synthesized two types of Ag-NPs capped with 3-mercaptopropionic acid as an anionic stabilizing agent (−46.1 mV zeta potential) and polylysine as a cationic stabilizing agent (40.2 mV zeta potential). In both cases, regardless of surface charge, a 5 orders of magnitude reduction in bacterial viability was observed.¹⁷ They suggested the need for further investigation and surface characterization to elucidate the reasons for the nonsensitivity of bacterial growth to Ag-NP surface charge.

The important role of hydrophobic groups in the organic coating is an additional factor that must be considered. Dutta et al.⁷⁴ studied the effects of different amphiphile coatings on the surface of Ag-NPs and found that the coating containing a hydrophobic group had a 5-fold lower minimum inhibitory concentration (i.e., higher toxicity) for *B. subtilis* species bacteria than Ag-NP coatings without hydrophobic groups. Although this same sizable effect was not demonstrated for other bacteria (*Micrococcus luteus* and *Staphylococcus aureus*) or fungi (*Candida albicans* and *Saccharomyces cerevisiae*),⁷⁴ this finding is consistent with that of another recent study showing that the relative hydrophobicity of polymeric coatings on Ag-NPs affects their attachment to hydrophobic surfaces, with greater attachment for more hydrophobic coatings.^{45,75} It is clear from these few studies that the roles of surface charge and organic coating type on Ag-NP interactions with environmental surfaces, including bacterial surfaces, and subsequent toxicity require more in depth investigation.

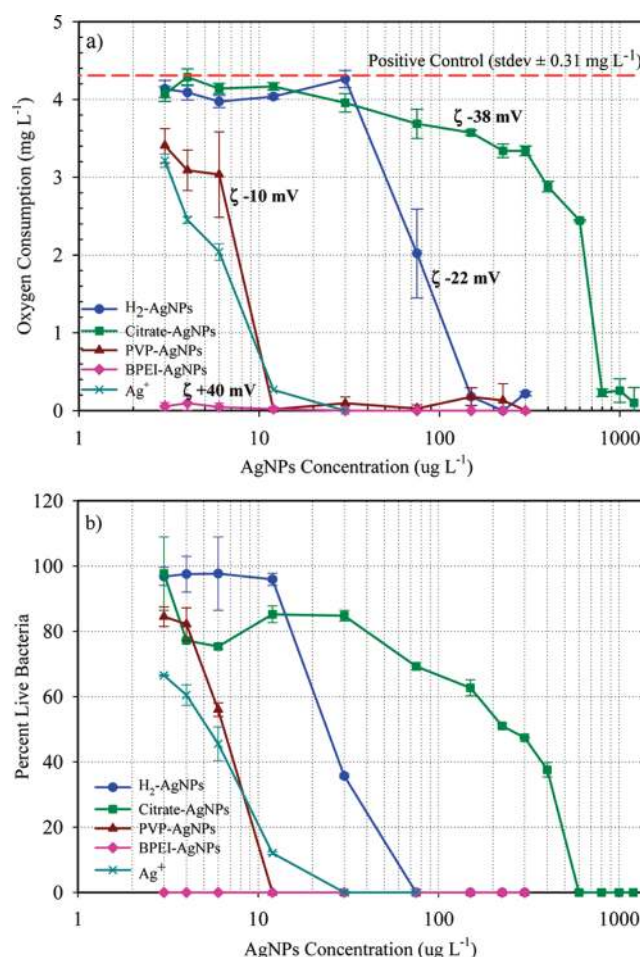


Figure 2. Inhibition of growth of Gram-positive bacteria as measured by oxygen consumption and live/dead stain by Ag-NPs having different surface charges. As the surface charge becomes more positive, the inhibition effect increases.⁶²

Another key component altering surface charge in environmental systems is NOM. Nanoparticles of any charge, especially positive, interact with and likely adsorb parts of these macromolecules. For example Fabrega et al.²¹ found that Suwannee River humic acid (a specific type of NOM) at 10 mg/L caused the Ag-NP surface to be more negatively charged than bare Ag-NPs, somewhat decreasing inhibitory effects of Ag-NPs on bacterial growth. Additionally, NOM plays a role in complexing Ag⁺, but these interactions have yet to be fully determined. Although these coating type effects have been investigated with Ag-NPs, further study requires a deeper understanding and more precise control of variables. This has been demonstrated effectively with both gold⁷⁶ and silica⁷⁷ NPs. These studies also highlight the importance of running appropriate “coating only” controls to eliminate any possible confounding toxic effects.

Particle surface charge is closely related to deposition and aggregation state because electrostatic and electrosteric forces prevent the particles from attaching to surfaces in the environment (i.e., particles with larger opposite charges will tend to aggregate less). Consequently, it is important to consider changes in particle aggregation state along with coating type as a factor controlling Ag-NP toxicity.

2.3. The Effect NP Aggregation State on Toxicity. There is growing evidence that small nanoparticles (i.e., < 20–

30 nm in diameter) can display properties significantly different relative to larger nanoparticles or bulk materials of the same composition.⁷⁸ It is also clear that nanoparticle surface area is predictably related to the amount of free ion released into suspension.⁷⁹ This relationship between surface area and ion release also transfers well to toxicity studies, where smaller sized particles have exhibited larger toxic responses.^{22,59,80} However, in many past studies designed to look for size-specific effects, size and state of aggregation were often measured only before experiments began, and the studies did not determine if nanoparticle size and/or aggregation state changed during the experiments. For example, the toxicity of Ag-NPs (coated with starch or bovine serum albumin) to zebra fish was tested on nanoparticles ranging in size from 5 to 20 nm as measured by transmission electron microscopy under ultrahigh vacuum conditions, but no characterization of the particles was done during or following exposure of the Ag-NPs to the experimental media,¹³ which is common practice.^{25,60,75,81} One recent study⁸² connected the aggregation state of citrate-stabilized Ag-NPs in a medium with toxic effects on *Daphnia* and concluded that the medium needs to be diluted at least 10-fold to reduce aggregation effects significantly.

Coated Ag-NPs can have toxic effects on nitrifying bacteria and fruit flies or have no effect on the microbial ecology of estuarine sediments. However, because we know little about the surface chemistry and aggregation state of these nanomaterials, little else can be said about the sources of enhanced or inhibited toxic effects compared with Ag ions. Nanoparticle aggregation will likely have an effect on toxicity by reducing the rate of dissolution, uptake by organisms, and stability of the nanoparticles against aggregation. Supporting this suggestion is a recent study by Reinsch et al.⁸³ which showed that aggregated Ag-NPs have a greater inhibitory effect on the growth of *E. coli* than dispersed Ag-NPs as a possible result of less complete sulfidation of the Ag-NPs in the aggregates.

Future work with Ag-NPs and environmental nanoparticles in general should consider aggregation state and surface charge as critical parameters to be measured before, during, and after experiments. If surface properties are important to consider when assessing the potential risk of Ag-NPs to the environment, transformation of the metallic core is also important to evaluate because it will strongly affect silver bioavailability.

3. SPECIATION OF AG IN DIFFERENT ENVIRONMENTAL SCENARIOS BASED ON THERMODYNAMIC CONSTRAINTS

Many organic and inorganic Ag-containing species can form and will affect the mobility, bioavailability, and toxicity of Ag-NPs and released Ag ions. Thermodynamic stability constants and solubility products for Ag-bearing inorganic compounds are good indicators of the potential inorganic phases that may precipitate in different natural environments (Table 1). It is

Table 1. Solubility Products (K_{sp}) of Silver-Containing Solids^{29,88}

compound	formula	K_{sp}
silver oxide	Ag ₂ O	4.00×10^{-11}
silver carbonate	Ag ₂ CO ₃	8.46×10^{-12}
silver chloride	AgCl	1.77×10^{-10}
silver sulfide	Ag ₂ S	5.92×10^{-51}
silver sulfate	Ag ₂ SO ₄	1.20×10^{-5}

important to note that the oxidation state for Ag is +1 for all the Ag-bearing inorganic compounds listed in Table 1. An oxidation step of the initial Ag⁰ core is therefore required to oxidize Ag⁰ to Ag⁺. The oxidation of silver is thermodynamically favored at room temperature ($\Delta G_{298}^0 = -11.25$ kJ/mol) and has been extensively studied both on single crystals and nanoparticles.^{63,84–86} (A more detailed discussion of surface oxidation can be found in section 4.1.)

Once oxidized, Ag⁺ tends to complex with soft bases such as sulfur as a type-B metal cation.⁸⁷ As expected, the solubility product of Ag₂S is very low (Table 1). Other potential reactions include the formation of AgCl and Ag₂CO₃ with similar solubility products (Table 1). However, the solubility constant is defined by the product of the activities of Ag⁺ and Cl[−] for AgCl and by the product of the activities of CO₃^{2−} and Ag⁺ squared for Ag₂CO₃.

AgCl is therefore more thermodynamically favored than Ag₂CO₃. As an example, if we consider a very simple aerobic aqueous system with a carbonate concentration of 0.03 mol/L, the concentration of Ag⁺ needed at pH 7 to start forming Ag₂CO₃ is 0.001 mol/L, which is much higher than the expected concentrations released in many environments. Under the same conditions (Cl concentration of 0.03 mol/L at pH 7), AgCl starts forming at an Ag⁺ concentration of 1×10^{-5} mol/L. The low stability of metal carbonates at pH < 8 makes the formation of a Ag₂CO₃ corrosion product unlikely and is not thermodynamically favored under normal environmental conditions. In contrast, the precipitation of AgCl is very likely in the absence of sulfide as described next. Finally, the formation of Ag₂SO₄ is not likely because it is not very stable compared with the previously mentioned precipitates. In addition to inorganic ligands, Ag-NPs or released Ag⁺ species can also bind strongly to organic matter. Comparison of the high stability constant of Ag(I)-organosulfur complexes with that of Ag(I)-carboxylate complexes illustrates the strong affinity of Ag(I) for sulfur (Table 2).⁸⁹

Table 2. Stability Constants (log K_f) for Ag⁺-Organic Compounds⁸⁹

compound	formula	log K_f
2-mercaptoethanol	HOCH ₂ CH ₂ SH	13.2
cysteine	-OOCCH(NH ₃ ⁺)CH ₂ SH	11.9
dimethyl sulfide	CH ₃ SCH ₃	3.7
methylamine	CH ₃ NH ₂	3.06
acetic acid	CH ₃ COOH	0.73
phenol	C ₆ H ₅ OH	0.34

Based on thermodynamic stability constants, the reactions of Ag-NPs and released Ag⁺ with Cl[−] and either inorganic HS[−] or organic thiols are therefore the most thermodynamically favored and environmentally relevant. It is important to note that while the presence of a capping agent may affect the kinetics of Ag-NP corrosion, it is doubtful that the thermodynamics of corrosion is altered because typical capping agents such as citrate and PVP³⁸ are much less reactive with the silver than sulfur-bearing natural phases. For example, the log K_f for a carboxylic group (acetic acid) is orders of magnitude lower than that of a thiol group (cysteine) (Table 2). In addition, we have shown that two different capping agents (PVP and GA) do not affect the observed equilibrium solubility of Ag-NPs.⁷⁹ The conditions that may favor the formation of one corrosion product over another are discussed in the

following sections considering different environmental scenarios.

3.1. Ag Speciation in Freshwater and Seawater. Based on thermodynamic constraints, Ag_2S and AgCl seem to be the more relevant inorganic species into which Ag-NPs will transform under environmental conditions. Speciation of silver will be dictated by the Eh-pH conditions of natural waters and solution composition and can be thermodynamically predicted for relatively simple systems. As an example, Figure 3 shows the

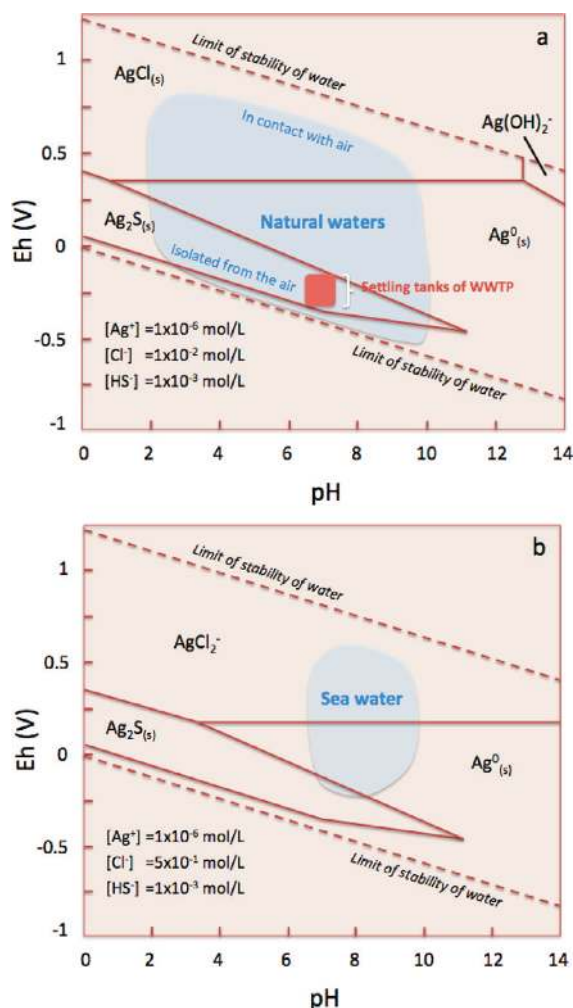


Figure 3. Eh-pH diagram for the system $\text{Ag-S-Cl-H}_2\text{O}$ at 25 °C and 1.013 bar calculated using Medusa⁹⁰ for two different Cl^- concentrations (solid phases are denoted with (s)). A more thorough description of the stability fields for natural waters (including seawater) and seawater can be found in Becking et al.⁹¹

Eh-pH diagrams for the system $\text{Ag-S-Cl-CO}_2\text{-H}_2\text{O}$ at 1×10^{-6} mol/L of Ag^+ , 1×10^{-3} mol/L of HS^- , 0.03 mol/L of CO_3^{2-} , and 0.01 mol/L Cl^- (typical concentrations for freshwater) (Figure 3a) and for the same system but a higher Cl^- concentration (0.5 mol/L), which is typical of seawater (Figure 3b). In the freshwater system, AgCl , Ag_2S , and Ag^0 are possible phases, depending on Eh, whereas in the seawater system, AgCl_2^- and Ag^0 are the most likely species. In both cases, Ag_2CO_3 as well as Ag_2O are not predicted to form. These phases are the thermodynamically expected Ag species formed in water, but slow transformation rates between phases may result in metastable phases. The identity of these phases will

have to be determined experimentally or by through characterization of field samples containing aged AgNPs. It is clear from these thermodynamic simulations that Ag speciation in natural waters will be strongly dependent on redox conditions more than pH. Under aerobic conditions silver chloride species are predicted to form. In this case Ag speciation depends on Cl^-/Ag ratio.

For very high Cl^-/Ag ratios typical of seawater (Figure 3b), soluble $\text{AgCl}_{(\text{aq})}$ or AgCl_2^- , the predominant species as well as AgCl_3^{2-} and AgCl_4^{3-} form. For lower Cl^-/Ag ratios typical of freshwater, $\text{AgCl}_{(\text{s})}$ may precipitate (Figure 3a). Under anaerobic conditions, precipitation of Ag_2S is expected.

It is important to note that these Eh-pH diagrams describe relatively simple systems that are usually not representative of natural systems because they do not account for interactions with biomass, NOM, clays, and other natural compounds. One can go a step further in these simulations by adding cysteine as a representative for thiol-containing organic ligands. The stability constant for Ag-cysteine is high ($\log K = 11.9$), and the presence of such complexes is therefore expected. The Eh-pH diagrams presented in Figure 4 were calculated assuming that thiols are present in aerobic waters when stabilized by

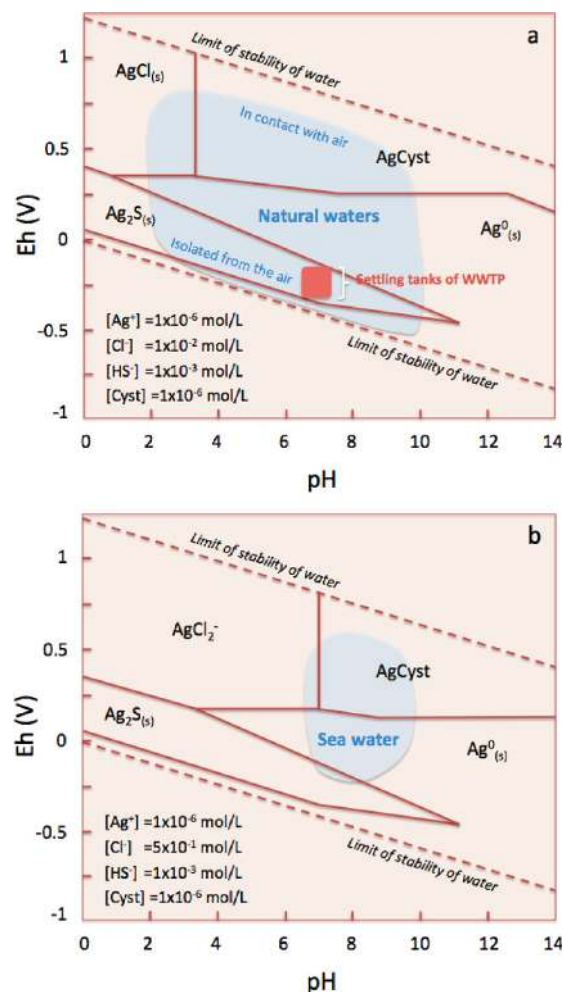


Figure 4. Eh-pH diagram for the system $\text{Ag-S-Cl-Cyst-H}_2\text{O}$ at 25 °C and 1.013 bar calculated using Medusa⁹⁰ for two different Cl^- concentrations (solid phases are denoted with (s)). A more thorough description of the stability fields for the natural water (including seawater) and seawater can be found in Becking et al.⁹¹

metals, potentially preventing or slowing down their oxidation.^{92–95} Stability constants for cysteine protonation and complexation with Ag (AgCyst and AgCyst₂[–]) were obtained from Adams and Kramer.⁹⁶ Under aerobic conditions, for equimolar concentrations of cysteine and silver (1 × 10^{–6} mol/L) and two different Cl concentrations, Ag strongly binds to cysteine (pH > 3.5 and pH > 7 for [Cl] = 0.01 and 0.5 mol/L, respectively) despite the fact that Cl is at a concentration 4–5 orders of magnitude higher than cysteine. As illustrated in these diagrams, Ag may strongly bind to both inorganic and organic S even in waters with high chloride concentrations. Thermodynamic simulations are useful for identifying potential environmental transformations of Ag-NPs. However, interactions with complex natural organic matter and the kinetics of those transformations can significantly alter those predictions. Of particular importance would be the oxidation of metallic AgNPs, which is the starting point of any environmental transformation. As discussed in the following sections, oxidation kinetics are a function of complex environmental conditions including redox and solution chemistries.

3.2. Fate of AgNPs in Wastewater Treatment Plants.

Models predicting the environmental loads of Ag-NPs suggest that most nanoparticles released from domestic and industrial sources will in most cases enter sewer systems and eventually WWTPs.^{54,55,97} For example, most of these nanoparticles can leach out of clothing in just a few washes.^{98,99} Little is known about the fate of Ag-NPs in WWTPs. However, much can be learned from previous investigations of the treatment of photoprocessing wastewaters enriched in Ag. First, WWTPs have proven to be very efficient at removing silver from treated water. As an example, Shafer et al.¹⁰⁰ showed that more than 94% of the Ag was trapped in biosolids. Second, photoprocessing wastewater did not affect treatment processes despite the high concentrations of ionic Ag⁺.^{101–105} For example, Pavlostathis and Maeng¹⁰² found that Ag had no effect on the performance of aerobic wastewater biodegradation for influent Ag concentrations of up to 1.85 mg/L. Most of the Ag was found to be associated with sludge solids, and the leachability of Ag from the resulting waste-activated sludge was 40 times lower than the regulatory limit. Initially bound to thiosulfate in the influent, Ag transforms into Ag₂S after moving through the activated sludge and sludge digestion processes of the WWTPs.^{103,105} This transformation is thermodynamically expected considering the pH and the redox conditions in the settling tanks (Figure 3a), especially in light of the high concentrations of sulfide in these types of facilities compared to natural waters. Similar to Ag present in photoprocessing wastewaters, sulfidation of both the Ag-NPs and the released Ag⁺ species in WWTPs is anticipated. Recently, Ag₂S-NPs were identified in the final-stage sewage sludge materials of a full-scale municipal wastewater treatment plant.¹⁰⁶ The authors of this study hypothesize that Ag₂S-NPs were formed during wastewater treatment by the reaction of either Ag-NPs or soluble Ag ions with reduced sulfur species. More recently, sulfidation of Ag-NPs was demonstrated in a pilot plant fed with municipal wastewater and spiked with Ag-NPs.¹⁰⁷ TEM analysis, X-ray absorption spectroscopy, and compositional analysis of the sludge showed that 85% of the Ag was sorbed to wastewater biosolids as Ag₂S, whereas only 5% of the Ag was found in the effluent after 43 days.¹⁰⁷ Silver in the effluent was also sulfidized to form Ag₂S. Ag-NPs will most likely be transformed into Ag₂S before entering WWTPs due to the relatively high (up to 6 mg/L) sulfide concentrations in sewer

pipes.¹⁰⁸ Human and industrial activities increase the generation of corrosive gas dissolved in water, including H₂S.¹⁰⁹ Furthermore, ppm-levels of dissolved H₂S have also been reported in rivers, seas, and brackish polluted aerobic waters.^{110–112}

In summary, the majority of Ag-NPs released into wastewater will most likely be transformed into Ag₂S and incorporated into sewage sludge. They then may re-enter the environment via application of sewage sludge to agricultural lands or from disposal of incinerator residues. The rate of sulfidation under environmentally relevant conditions should be determined as well as the stability of Ag₂S against oxidation of S and subsequent release of Ag ions.

4. AG-NP CORE TRANSFORMATIONS AND EFFECT ON TOXICITY

The formation of all environmentally relevant Ag-species described in section 3 from metallic silver has been partly studied as has the impact of such transformations on toxicity. The main transformations are discussed in more depth in the following sections.

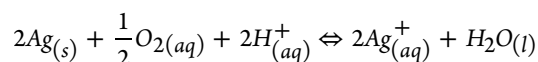
4.1. Oxidation of Bulk Ag and Ag-NPs. Although silver oxide does not seem to be thermodynamically favored in the presence of chloride and sulfur in natural waters (Figures 3 and 4), it may precipitate when exposed to the atmosphere, where the amount of O₂ is an order of magnitude higher than S- or Cl-bearing corrosive gases such as H₂S or Cl₂. When exposed to oxygen, silver reacts to form a silver oxide (Ag₂O) surface layer. The oxidation of silver is thermodynamically favored at room temperature ($\Delta G_{298}^0 = -11.25$ kJ/mol) and has been extensively studied on single crystals mainly for catalysis purposes.^{85,86,113} The oxidation of silver surfaces has also been observed in the case of Ag-NPs despite the presence of organic surfactants.^{63,84,114–116} Detection of an Ag₂O layer on the surface of Ag-NPs is challenging with available spectroscopic tools because oxygen is typically also present in organic water-soluble surfactants. Silver oxidation has mainly been observed using UV–vis spectroscopy because it causes a red-shift of the surface plasmon band.^{63,84,114–116} Silver oxidation has also been observed by *in situ* Raman spectroscopy¹¹⁷ and X-ray photoelectron spectroscopy.¹¹⁶ Oxidation reactions are thermodynamically enhanced as silver particle size decreases.¹¹⁶ The standard formation free energy value ($\Delta G_{298}^0(r)$ in kJ/mol) is inversely proportional to the particle's radius of curvature (r in nanometers)

$$\Delta G_{298}^0(r) = -11.25 - \frac{57.5}{r} \quad (1)$$

In addition, very small Ag-NPs (<2–3 nm) have been shown to be more susceptible to oxidation than bulk silver because of lower redox potentials.^{118–120} Aside from clearly favorable thermodynamics, little is known about the kinetics of oxidation of AgNPs in complex environments. There is a need for further investigation of the key parameters that control the kinetics and extent of oxidation (particle size and surface energy, nature and concentration of the oxidant, humidity, nature of the coating...).

4.1.1. Solubility Implications. The dissolution of Ag-NPs to form Ag ions is one of the main environmental risk issues. When assessing the solubility of silver under aerobic conditions, one has to consider not only metallic silver, which is insoluble in anaerobic waters, but also the possible presence of an oxide layer (Ag₂O) on the surface of the nanoparticle.^{28,61,82,114–116} In contrast to metallic silver, silver oxide dissolves in pure water

($K_{sp} = 4 \times 10^{-11}$),¹²¹ which results in the release of Ag^+ cations. Once the Ag_2O layer is dissolved, further oxidation/dissolution of metallic Ag-NPs occurs only in the presence of an oxidant in solution. Liu et al.³² found that removing dissolved oxygen from water completely inhibits the release of dissolved Ag ions, which shows the role of O_2 in surface oxidation. In the presence of dissolved O_2 , Ag^+ solubility is enhanced when pH is decreased, demonstrating the importance of protons.^{32,122} The following reaction was proposed for the dissolution of metallic silver in a simple aerobic, acidic aqueous solution



Dissolution rate is strongly dependent on particle size. As an example, the dissolution rate for 5.4 nm Ag-NPs in the presence of a strong oxidant (H_2O_2) is about 9 times faster than that for 20.5 nm Ag-NPs.¹²³ Similarly, Liu et al. have shown that the dissolution rate constant, normalized by the surface area, varies by about 1 order of magnitude for AgNPs 4.8 nm in diameter compared to a silver foil.²⁹ In summary, in the presence of oxygen, Ag_2O forms on the Ag-NP surface and Ag^+ subsequently dissolves into aqueous solution. In an environmentally relevant pH range, dissolution is enhanced at low pH. Finally, kinetic and thermodynamic data show that both oxidation and dissolution are enhanced as particle size decreases.

4.1.2. Impact of Oxidation on Toxicity. Lok et al.⁵⁹ showed that 9.2 nm Ag-NPs prepared under reducing conditions (no Ag_2O surface coating) are not toxic to *E. coli* under anaerobic conditions. In contrast, after bubbling aqueous solutions containing Ag-NPs with oxygen for 30 min before exposure to *E. coli*, the same particles (with an Ag_2O surface coating) showed a strong deleterious effect on *E. coli* colony formation under the same exposure conditions. Similarly, Gunawan et al. have shown the influence of oxidation state of Ag on antimicrobial action. By tuning the oxidation state of AgNPs using an optically selective approach, they were able to tune the antimicrobial activity of the Ag-NPs.¹²⁴ These results support those from similar experiments on bulk silver.^{125,126} In these experiments, the presence of a soluble Ag_2O layer was the key to silver exhibiting antimicrobial properties. When possible, toxicity studies performed under both aerobic and anaerobic conditions can be useful for determining the concentration of dissolved Ag ions required for toxicity and to isolate possible nanoparticle effects.

Oxidation of Ag-NPs and subsequent dissolution to form Ag ions have been shown to be important causes of the observed toxicity of Ag-NPs to different organisms, as previously discussed. The aerobic conditions that Ag-NPs encounter in many environments result in the oxidation and release of Ag ions. Once released in the environment, Ag ions play a major role in toxicity, and environmental conditions such as solution pH and redox conditions that control Ag-NP oxidation and dissolution need to be carefully characterized. In addition to pH and redox conditions, however, other variables that affect the behavior of Ag-NPs, including solution ionic strength, specific ions and ligands, and natural organic matter, should be considered.

4.2. Reaction of Ag with Chloride Ions. The reaction of Ag with chloride has been studied in different contexts such as for making thin AgCl films on silver surfaces with unique catalytic and electronic properties.^{127–129} Silver chloride nucleation on Ag (111) surfaces was shown to preferentially

occur on atomic steps.¹³⁰ Assuming that edges facilitate nucleation, one can reasonably assume that nanoparticles will react more readily with chloride because the surface of a nanoparticle can be viewed as a continuous series of edges and steps. Another field of investigation is related to the formation of specific Ag-NP structures, such as a disk-like structure, by selective etching of silver nanoprisms caused by chloride.¹³¹ Chloride as well as halides in general have been used to control Ag-NPs shape by selective etching.^{132,133}

From an environmental point of view, dissolution of Ag-NPs in the presence of chloride in aqueous solution has not been thoroughly investigated. It is important to note that an initial oxidation step of the metallic silver surface is a prerequisite before chloride can interact with oxidized silver.¹³⁴ The dissolution behavior of Ag-NPs in the presence of Cl^- ions was shown to be inversely correlated with the Ag/Cl ratio (i.e., decreasing Ag/Cl increases dissolution).⁶³ This is the case because relatively small amounts of Cl^- may scavenge toxic Ag^+ from solution, forming AgCl precipitates, whereas increasingly higher levels of Cl^- may form bioavailable anionic Ag complexes such as AgCl_2^- , AgCl_3^{2-} , and AgCl_4^{3-} , possibly increasing the toxicity to both sensitive and resistant bacteria.^{135,136} The dissolution rate of Ag-NPs in aerobic systems and in the presence of Cl^- is, therefore, difficult to predict because the formation of AgCl shells on the surfaces of Ag-NPs or dissolution and formation of $\text{AgCl}_x^{(x-1)-}$ could make the dissolution rate a complicated function of Cl^- concentration. For example, Ho et al. showed that the presence of Cl^- ions in solution can completely inhibit or severely decrease the kinetics of dissolution of Ag-NPs.¹⁰⁸ They postulated that relatively insoluble AgCl precipitates on the surface of the Ag-NPs, limiting particle dissolution (and likely penetration of oxygen and further oxidation of the Ag(0) substrate). A recent study by our group provides evidence for the precipitation of AgCl both as separate phase and associated with Ag-NP surfaces.³¹

The effect of Cl^- anions on the uptake and toxicity of Ag^+ has been investigated. For example, toxicity of Ag^+ in presence of Cl^- among various species of fish has been studied and appears to be dependent on different mechanisms of Cl^- uptake among different fish species.^{137,138} More recently, the toxicity of AgCl colloids has been compared to Ag-NPs and Ag ions. Choi et al.⁸¹ have shown that the inhibition of nitrifying bacterial growth by AgCl colloids can be as significant as Ag ion inhibition but less than 14 nm AgNPs. However, such a comparison cannot be generalized to AgNPs because the toxicity of AgNPs will strongly depend on the oxidation/dissolution rate of the surface which is size dependent as discussed earlier.

4.3. Reaction of Ag with Sulfur. Sulfidation of Ag surfaces in contact with various S-bearing gases (H_2S , SO_2 , OCS , and CS_2)^{139,140} has been studied extensively in part because sulfidation can impact the thermal and electrical conductivity of silver used in electrical equipment. It was found that in dry laboratory air, where the measured concentration of H_2S was less than 0.2 ppb, a Ag_2S layer begins to grow within an hour.¹⁴¹ Performing the same experiment in a dry nitrogen atmosphere completely inhibits this reaction, demonstrating the crucial role of O_2 as an electron acceptor.^{141,142} Water has also been shown to strongly enhance the sulfidation process.^{128,139,143} One possible explanation for this behavior is that given the high solubility of H_2S in water, it is acting as an appropriate intermediary medium, increasing the contact

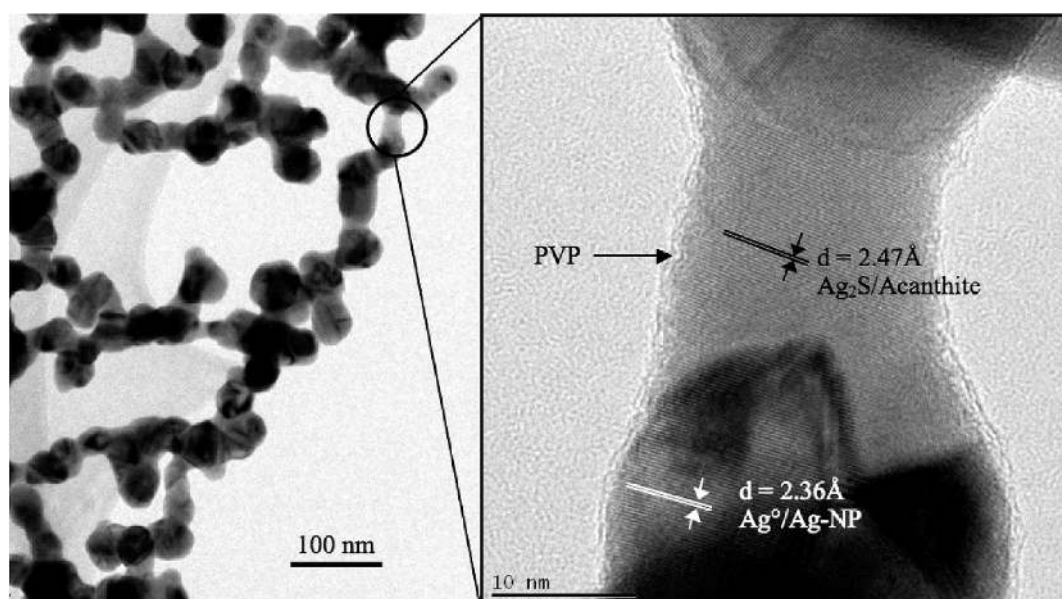


Figure 5. TEM images of partly sulfidized Ag-NPs. The right image is at higher magnification and is centered on one of the nanobridges observed at low magnification (left image). From Levard et al.³⁰

probability between the Ag^0 surface and H_2S . Similar to reactions with chloride, sulfidation kinetics partly depend on the surface orientation and the presence of steps, which facilitates nucleation.¹⁴¹ Due to their presumed edge-dominated surface structure, Ag-NPs may sulfidize faster than bulk Ag. However, sulfidation of Ag-NPs is not well understood at a fundamental level despite the fact that it may strongly affect their properties in the environment. McMahon et al.¹⁴⁴ showed that bare Ag-NPs are rapidly sulfidized in ambient laboratory air. The sulfidation rate, measured by Auger and UV-vis spectroscopy, was found to be 7.5 times higher than that of bulk silver under the same conditions.¹⁴⁵ Under similar conditions (laboratory air), PVP-coated nanowire Ag gradually transformed into Ag_2S .¹⁴⁶

From an environmental perspective, it is crucial to study sulfidation processes not only in air but also in water because the majority of Ag-NPs may end up in sewage pipes and finally in wastewater treatment plant effluent and biosolids.^{54,55,97} Previously, we investigated the sulfidation of PVP-coated Ag-NPs in water in contact with HS^- .³⁰ TEM observations show that Ag_2S forms nanobridges between the Ag-NPs (Figure 5), leading to chain-like fractal structures that suggest silver surfaces are first oxidized, partially dissolved, and reprecipitated as Ag_2S nanobridges between Ag-NPs. The use of synchrotron-based techniques has shown that about 2/3 of the Ag_2S formed is amorphous (undetectable using laboratory-based X-ray diffraction). A similar study has provided evidence for two different mechanisms of sulfidation, depending on sulfide concentration.¹⁴⁷ At high sulfide concentrations, sulfidation occurs by direct conversion of Ag-NPs to Ag_2S -NPs through a solid-fluid reaction, whereas at lower sulfide concentrations, oxidative dissolution and precipitations seems to be prevalent. This is an important piece of information since in the natural environment, sulfide might be present at a relatively low concentration compared with laboratory experiments that favor the second phenomena (i.e., the release of free, potentially toxic, Ag^+ before it precipitates into Ag_2S). Transformation mechanisms, such as the formation of Ag_2S nanobridges, might not be relevant to what occurs in natural waters where both

sulfide and AgNPs are present at very low concentrations and competing with many others ligands. Although batch experiments are a first step toward understanding how sulfidation processes affect AgNPs properties, additional experiments need to be performed in more realistic natural systems to identify the intermediate products and their potential impact on ecosystems.

Free sulfide, such as HS^- , is not the only source of sulfide that can react with Ag. Laboratory experiments have shown that Ag ions quickly replaced Zn ions in Zn-sulfide complexes probably because of the higher stability of Ag_2S compared with ZnS .¹⁴⁸ Similar behavior has been reported for Ag ions in contact with FeS .¹⁴⁹ Moreover, Cl^- , Cu^{2+} , and dissolved organic matter showed no effect on the Ag-FeS system. Only the presence of organo-sulfur (thiol) ligands resulted in increased dissolution of Ag^0 . Indeed, Ag ions bind strongly with organosulfur compounds, with the greatest affinity for thiol-containing ligands. Organosulfur compounds are ubiquitous in nature and are produced by aquatic organisms during oxidative stress or exposure to toxic metals. Adams and Kramer¹⁵⁰ concluded that due to the formation of extraordinarily stable sulfide complexes, silver should outcompete other metals for the available sulfide; the exception is Hg because it forms an even more stable complex with sulfur ($K_{\text{sp}}(\text{HgS}) = \sim 10^{-53}$).¹⁵¹ Silver(I) thiolates also react rapidly with H_2S or HS^- ligands to form Ag_2S . The reverse reaction is unlikely because of the high stability of Ag_2S .⁸⁹ However, thiols such as cysteine that can strongly bind to Ag-NPs may affect rates of sulfidation on time scales that are relevant to environmental behavior and effects.

Although sulfidation is expected under anaerobic conditions, metal sulfides have been found in aerobic waters at nM to μM concentrations.^{148,152} Under these conditions, previous studies have demonstrated that heavy metal ions such as Hg^{2+} , Ag^+ , Cd^{2+} , Zn^{2+} , Cu^{2+} , and Pb^{2+} can form stable complexes with inorganic sulfides and thiols and potentially prevent or slow down the oxidation of sulfides and thiols in oxic waters.^{92–95} Thus, glutathione (GSH) complexed to Ag^+ ($\text{Ag}(\text{GSH})$) was found to be completely stable in the presence of H_2O_2 in the

time frame of the experiment (2 days), whereas GSH alone oxidized within minutes.

In summary, Ag-NPs and their released Ag species react strongly with sulfur in ambient air as well as in aqueous solutions under both aerobic and anaerobic conditions. The sources of sulfur are wide-ranging, from sulfur-bearing gas to organic species to metal sulfide minerals having a lower stability than Ag_2S (i.e., ZnS , FeS , PbS , and CuS). An important consequence of the reaction of Ag with sulfur is its effect on the bioavailability and toxicity of Ag^+ in the environment. For example, Bianchini et al.^{153,154} showed that the acute toxicity of Ag^+ to *Daphnia magna* was reduced by about 5.5-fold in the presence of environmentally relevant levels of sulfide. Because release of toxic Ag^+ is thought to be one of the main mechanisms governing Ag-NP toxicity, one therefore expects that Ag-NP toxicity will be lowered in the presence of sulfur because of the formation of relatively insoluble Ag_2S . For example, we have demonstrated that in the presence of a small amount of sulfur ($n_{\text{S}}/n_{\text{Ag}} = 0.019$), representing the amount needed to form a monolayer of Ag_2S on 35-nm Ag-NPs, solubility was reduced by about 7-fold.³⁰ For higher levels of sulfur ($n_{\text{S}}/n_{\text{Ag}} > 0.432$), no Ag^+ was detected in solution after one month.³⁰ Another example supporting this hypothesis is a study of the toxicity of Ag-NPs and Ag^+ to nitrifying bacteria isolated from wastewater treatment plants. Toxicity was reduced by up to 80% in the presence of sulfur.^{155,156} Finally, Suresh et al. (2011) have shown that 9 nm Ag_2S nanoparticles produced by biosynthesis were noninhibitory and noncytotoxic to Gram-negative (*Escherichia coli* and *Shewanella oneidensis*) and Gram-positive (*Bacillus subtilis*) bacterial systems as well as eukaryotic cell lines including mouse lung epithelial (C 10) and macrophage (RAW-264.7) cells.¹⁵⁷ Although the formation of Ag_2S seems to reduce acute toxicity, depending on organisms and their diet, it can remain bioavailable and have long-term impacts on the organism's functionalities such as reproduction.¹⁵⁸

5. OPEN QUESTIONS AND NEEDED RESEARCH

We have reviewed important transformation processes of Ag-NPs that need to be considered when addressing their toxicity in the environment and have also discussed the surface properties and phase transformations of Ag-NPs. Although surface properties are crucial in understanding the environmental behavior of Ag-NPs in terms of their stability against aggregation, mobility, and toxicity, phase transformations of Ag-NPs have the largest impact on their fate and also lead to modification of their surface properties. The relationship between surface properties of fresh Ag-NPs and their environmental behavior has been only partly explored, as discussed in section 2. Moreover, the surface properties of newly formed transformation products, especially nanosized Ag_2S , and their associations with NOM, are currently unknown. Surface properties of transformed Ag-NPs need to be investigated in more depth to predict the stability of corrosion products over time.

Due to oxidation and the strong affinity of Ag metal for O, Cl, S, and organic compounds, particularly those containing thiol groups, the lifetime of Ag-NPs in the environment is likely to be relatively short. This is especially true for Ag-NPs as compared to bulk silver because the kinetics of corrosion increase as particle size decreases. Sulfidation is the most likely corrosion process that metallic silver will undergo because of the very high stability of Ag_2S and the significant amount of

reactive sulfide present in polluted water and in WWTPs. Although we are beginning to understand how these particles transform and how transformations affect their properties, a number of important questions need to be addressed to better predict the environmental fate of Ag-NPs. Five important questions have been identified and are discussed below.

5.1. Assessing the Stability and Toxicity of the Nanosized Ag_2S . Figure 6 illustrates important unanswered

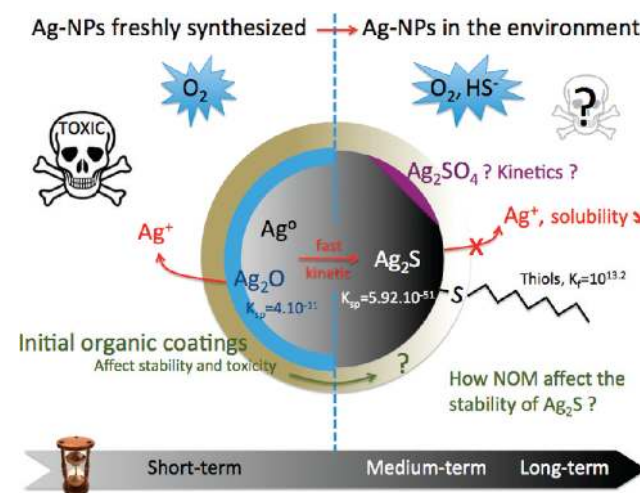


Figure 6. Current knowledge (and unknowns) about environmental transformations of Ag-NPs.

questions concerning the stability of nanosized Ag_2S . In particular, how does NOM affect the stability of Ag_2S nanoparticles once biosolids or sewage sludge containing these nanoparticles is spread on agricultural fields? For example, humic acids have been shown to reduce bulk Ag_2S solubility by about 75%, whereas both cysteine and thiosulfate almost completely inhibit Ag release into solution.¹⁵⁹ However, to the best of our knowledge, systematic investigations of the effects of these ligands on nanosized Ag_2S have not been undertaken. Moreover, the mechanisms and kinetics of oxidation of Ag_2S -NPs in contact with air or as a result of microbial transformation to Ag_2SO_4 need to be addressed as Ag_2SO_4 is considerably more soluble than Ag_2S ($K_{\text{sp}} = 1.2 \times 10^{-5}$ and 5.92×10^{-51} , respectively).

Another open question concerns the toxicity of Ag_2S -NPs, which show limited acute toxicity compared to unsulfidized Ag-NPs because sulfidation significantly decreases the solubility and availability of Ag ions. However, because of the potential accumulation and long-term stability of Ag_2S -NPs in the environment, chronic exposure effects need to be examined as well. Toxicity studies (acute and chronic) for partially or entirely sulfidized Ag-NPs (Ag_2S -NPs) on different organisms should be carried out to determine how complete or partial sulfidation of Ag-NPs affects toxicity. Characterizing the effect of partial sulfidation on toxicity might be important for situations where the amount of sulfide is low compared to Ag concentration.

5.2. Characterizing Ag-NP Transformations under More Realistic Environmental Conditions. Most of the studies discussed in this review focus on a specific Ag-NP-ligand interaction at relatively high Ag concentrations. For example, sulfidation processes have been studied in batch experiments with high concentrations of sulfur and Ag-NPs.^{30,147} However, we know little about the rate of sulfidation

of Ag-NPs in environments with limited sulfide and oxygen and lower Ag-NP concentration. We also know little about the effects of potential competing natural ligands, such as NOM, which can prevent or slow down the sulfidation process. In this regard, microcosm- and mesocosm-type experiments are needed to better simulate environmental conditions.

5.3. The Core–Shell Structure of Ag-NPs. Most studies focusing on transformations of Ag-NPs consider the metallic core and the organic shell separately and almost never consider the core–shell structure as a whole. One can expect that transformation of the Ag⁰ core will have an impact on the stability of the coating, causing desorption or oxidation, for example. The reverse is also true, and thus very little is known about how the organic coating affects dissolution rates of the metallic core. Another question concerns how the coating affects transformation rates of the metallic core. One can expect that the organic coating can act as a passivating layer inhibiting or limiting the interaction of the Ag-NP surfaces with other ligands. Interfacial reactions between the surface of the metallic core and the organic coatings have not been thoroughly investigated despite the fact that they may strongly affect the behavior of core–shell type particles in the environment.

5.4. Identifying Ag-NP Shape Effects on Reactivity and the Kinetics of Transformations. Morphology is clearly an important parameter that will govern the surface reactivity and therefore the fate of Ag-NPs in the environment. Although nanoparticle size is often taken into consideration when assessing reactivity or toxicity, very few studies have focused on nanoparticle shape. One such study showed that Ag-NPs undergo a shape-dependent interaction with *E. coli* because of differences in reactivity of different crystallographic facets.¹⁶⁰ Indeed, it is well-known that different crystallographic surfaces of bulk solids have different reactivities, which has been extensively explored in heterogeneous catalysis.^{161,162} However, it is important to note that shape is probably not the only factor that affected toxicity in this particular study. Particle size varies along with shape, and this affects the total dissolution rate of the particles. Because the authors do not provide dissolution data, it is difficult to attribute the observed toxicity exclusively to shape effects. Differences in surface reactivity are also used to control the synthesis of nanorods. For example, small carboxylic acids such as citric acid preferentially sorb on Ag(111) rather than Ag(100), allowing growth in only one direction.¹⁶³ In this regard, one would expect that the reactivity of Ag-NPs and transformation rates of Ag-NPs with (in)organic ligands in the environment will depend on both nanoparticle size and shape.

5.5. The Surface Structure of Ag-NPs. Although the surface structure and surface composition of Ag-NPs control properties such as solubility and reactivity, surface structure is a major unknown because there are currently no methods available for determining nanoparticle surface structure at the molecular level. Classical macroscopic models have been used to provide useful guidance in understanding Ag-NP properties. As an example, the modified form of the Kelvin equation was recently used to predict the solubilities of a series of Ag-NPs ranging from 5 to 80 nm in size.⁷⁹ Solubility (equilibrium) was shown to be independent of the nature of the pristine organic coating or the synthesis method. However, this model is valid only in the case where surface stress is constant as a function of particle size. Surface stress, which is partly defined by surface strain, is expected to change significantly for very small particles, preventing the use of such a model. Ultimately, a detailed knowledge of surface structure (and surface

composition) is needed to be able to fully understand important processes governing the fate of Ag-NPs in the environment such as aggregation and dissolution.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was supported by the National Science Foundation (NSF) and the Environmental Protection Agency (EPA) under NSF Cooperative Agreement EF-0830093, through the Center for Environmental Implications of Nanotechnology (CEINT). Any opinions, findings, conclusions, or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NSF or the EPA. This work has not been subjected to EPA review and no official endorsement should be inferred.

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