The Role of Sulfhydryl-Containing Low Molecular Weight Ligands for the Environmental Fate of Zinc Sulfide and Metallic Silver Nanoparticles

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Civil and Environmental Engineering in the Graduate School of Duke University

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ABSTRACT

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Abstract

Nanomaterials often exhibit enhanced reactivity relative to their larger colloidal counterparts because of the high specific surface area and number of imperfections on the crystal lattice at the nanoscale. Understanding the environmental factors that control the reactivity and bioavailability of natural and manufactured nanomaterials is important for ecosystems management, contaminated water remediation, and the assessment of potential risks from the industrial use on nanomaterials. Dissolved organic matter (DOM) acts as a moderator of reactivity and bioavailability for dissolved and particulate moieties in natural waters. DOM consists of a range of low and high molecular weight species that are complex and heterogeneous. It has been historically categorized based on operational definitions, rather than physicochemical properties. In order to understand the effect of DOM on nanomaterials, there is an urgent need to study how specific properties of DOM, such as ligand groups, may interact with the nanomaterials.

The goal of this research was to study how cysteine, a low molecular weight metal-binding ligand, affects the composition and reactivity of nanoparticulate zinc sulfide and metallic silver. Zinc sulfide was used as an example of nanoparticulate metal sulfides which occur naturally in sulfidic environments. Metallic silver nanoparticles
were used as an example of manufactured nanoparticles, because of thier wide use in consumer products. Both types of nanomaterials contain metal constituents (zinc and silver) that are expected to strongly bind to sulfhydryl-containing ligands (such as cysteine) in the environment. Serine is structurally similar to cysteine, with the only difference of a hydroxyl group in the place of the sulfhydryl group of cysteine. Therefore, serine was used for comparison as a hydroxyl-containing analogue to cysteine.

The aggregation kinetics of zinc and other metal sulfide nanoparticles in the presence of cysteine and serine were investigated using dynamic light scattering. Cysteine decreased aggregation rates of the particles, while serine had no effect on their aggregation behavior. Further experiments revealed that the mechanism of stabilization occurred through the adsorption of cysteine on zinc sulfide, which induced electrostatic charge on the particles surface. A direct link was established between the amount of cysteine sorbed and attachment efficiency, an indicator of the tendency of particles to aggregate. These results shed light on discrepancies in the literature between metal sulfide precipitation experiments conducted in our lab and work on the formation and aggregation of zinc sulfide nanoparticles on biofilms of sulfate reducing bacteria.

The early-stage growth and aggregation kinetics of zinc sulfide nanoclusters in the presence of cysteine was studied in detail using a suite of complementary techniques. Growth and aggregation experiments have been traditionally difficult to
study due to instrumental imprecision, but newly developed analytical tools and software products have made it possible to study the early-stage formation of nanoclusters. Experiments with small angle X-ray scattering, X-ray diffraction, dynamic light scattering, and X-ray absorption spectroscopy at the extended fine structure range showed that cysteine controlled the growth and aggregation of zinc sulfide nanoclusters. The molar ratio between zinc, sulfide, and cysteine was a determining factor in the precipitation process. When zinc and sulfide were in equimolar concentrations with cysteine, very small nanoclusters of about 2.5 nm formed within 12 hours and aggregated to structures with hydrodynamic diameter larger than 100 nm. When cysteine was in excess of zinc and sulfide, aggregation was held to a minimum, but monomer nanoclusters were able to grow to about 5 nm in 12 hours. Overall, these results indicate the importance of thiol ligands on the growth, aggregation, and aggregate structure of metal sulfides.

The effect of metal ligands on metal-based particle surfaces is of particular interest for manufactured nanoparticles, because they are typically coated with an organic coating during the production process. These coatings are sorbed on the particles surface and are likely to interfere between the metallic surface and ligands in DOM. Dissolution experiments using citrate and polyvinylpyrrolidone (PVP) coated zero valent silver nanoparticles in the presence of cysteine and serine showed that cysteine dissolved both types of particles, while serine did not. Dissolution rates
depended on the aggregation state of the particles exposed to cysteine. As indicated by zeta potential and adsorption measurements, cysteine replaced the coating on the particles surface and altered their aggregation pattern. X-ray absorption spectroscopy near the absorption edge showed partial oxidation of silver and formation of Ag(+)I-sulfur bonds, indicating that the thiol group in cysteine formed chemical bonds with oxidized surface silver atoms. A comparison between the two coatings showed that citrate coated particles dissolved approximately three times faster than PVP coated particles. Overall, these results show that metal binding ligands can drastically change the fate of manufactured silver nanoparticles in the environment and that this effect is moderated by surface coatings.

The results of this study suggest that cysteine, a metal binding ligand was able to induce and control transformations, such as growth, aggregation, dissolution, and surface reactivity of zinc sulfide and metallic silver nanoparticles. Cysteine adsorbed on metal sites on both ZnS and Ag particles, inducing changes on their surface charge. Aggregation kinetics of ZnS particles decreased because of a net decrease in zeta potential compared to the bare particles. On the contrary, cysteine enhanced the aggregation of Ag particles, by replacing the citrate and PVP coatings on the particles surface. Finally, cysteine-Ag(+)I bonds caused strong polarization on the particles surface and lead to oxidative dissolution of the particles.
Overall, this research provides a better understanding of the fate of natural and manufactured nanoparticles in anaerobic waters, where thiols are present in significant amounts. It may also be used for risk assessment of manufactured nanomaterials and the production of safer and environmentally responsible materials.
In memory of uncle Harry

and

to my parents
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Chapter 1. Introduction

1.1 Motivation

Nanoparticles are considered to be colloids with at least one dimension in the range of 1 and 100 nm. Due to their small size, they can resist settling for extended times and travel longer distances than larger colloidal particles. In addition, they often exhibit properties that are different from their bulk counterparts, such as enhanced reactivity [1]. Recent developments in analytical methods have allowed scientists to study nanoparticles, which have historically been considered as dissolved species because they pass through filters that were used to operationally define dissolved and particulate fractions in water [2]. These tools allowed for a better understanding of processes such as mineral weathering, mineral-bacteria interactions, and metal transport in acid mine drainage systems [3-5]. For example, the study of biotic or abiotic formation of particles with metal constituents and the adsorption of metals on particle surfaces was made possible with the development of synchrotron-based techniques and electron microscopy (e.g. [6, 7]). The detection of metal sulfide nanoparticles in acid mine drainage waters with electron microscopy demonstrated the mobility of zinc sulfide nanoparticles and their ability to carry other toxic metals, such as Pb and As [8].

The formation of mineral sulfide colloids and nanoparticles is important for the aquatic fate and transport of toxic metals, such as zinc and mercury in sediment pore water, wastewater effluent, and other anaerobic environments [9-13]. Although these
particles form in anoxic waters, they may enter oxic waters and remain stable for days [14, 15]. Therefore, studying the processes that lead to their formation and persistence can be useful for understanding the biogeochemical cycle of these elements and for restoring contaminated ecosystems.

The lack of knowledge about the processes governing the fate and transport of natural nanoparticles poses a barrier for assessing the environmental impact and possible harm from the emerging industry of nanomaterials [16]. The number of consumer products with nanomaterials has been rapidly increasing since they first appeared at the beginning of the century [17]. In many cases nanomaterials get in direct contact with humans (skin, stomach, hair, etc.) and are readily discharged in wastewater or natural aquifers [18]. Because of its antimicrobial activities, silver is widely used in nanomaterials and is present in products such as personal care items, clothing, cosmetics, sporting goods, and sunscreens. Although silver containing nanoparticles are not new in nature and consumer products, little is known of their fate in the environment [19, 20]. The same processes that determine the fate of naturally-occurring metal bearing nanoparticles are also likely to determine the fate of manufactured nanoparticles.

Transport, reactivity, and bioavailability of colloidal particles are important processes for the fate and transport of metals in natural waters and are often controlled by dissolved organic matter (DOM). Naturally-occurring and manufactured
nanoparticles may form larger aggregates that settle out of the water column. However, this process can be slowed by DOM, which is capable of stabilizing colloidal particles in suspension (e.g. [21, 22]). In addition, organic matter may enhance the dissolution of particles [9]. Organic molecules adsorb on particle surfaces through hydrophobic interactions or specific chemical bonding (e.g. [23, 24]). However, the mechanisms of interactions between DOM and colloids are poorly understood. The greatest obstacle is the diversity and complexity of the molecules that comprise DOM.

Ligand groups, such as carboxyls (–COOH), hydroxyls (–OH), amines (–NH₂), and thiols (–SH) in DOM react with dissolved metal ions and metal constituents of suspended particles. The extent to which these reactions occur depends on the concentrations and reactivity of the ligands. The concentrations of ligand groups in DOM generally follows the order: –COOH,–OH > –NH₂ > –SH. Thiols can be up to four orders of magnitude less concentrated than carboxyls [25]. However, thiols have up to eight orders of magnitude higher formation constants with soft metals such as Zn²⁺, Ag⁺, and Hg²⁺ that are typically at trace levels (i.e. less than the concentration of strong ligand binding sites such as sulfhydryl). Therefore, thiols can be important for the speciation and bioavailability of these metals in natural waters [26, 27]. The scientific knowledge on the reactivity of ligand groups with dissolved metals has expanded over the years, but little is known of their reactivity with metal-based particles. Recent studies have demonstrated that thiol containing ligands decrease the kinetics of precipitation and
aggregation of ZnS and HgS [28, 29]. These findings indicate that thiol ligands may play a key role for the fate of particles with soft metal constituents. In addition to precipitation, other processes such as surface reactions and dissolution work in synergistic or antagonistic ways and ultimately determine the particles fate.

The objective of this dissertation was to assess how metal-binding ligands influence the reactivity of metal-based nanomaterials in the environment. Cysteine was used as a model compound for low molecular weight sulfhydryl organic ligands. ZnS nanoparticles were used as an example of naturally-occurring metal sulfides. Metallic silver nanoparticles were used as an example of metal-based manufactured nanomaterials. The reactivity of ZnS and Ag nanoparticles towards cysteine was determined by studying the formation, surface composition, solubility, and aggregation of these nanoparticles. Studying the reactivity of metal-based nanoparticles towards specific ligand groups may help scientists predict the particles fate in the environment and direct the nanomaterial industry towards safer and environmentally responsible products.

1.2 Zinc sulfide nanoparticles

Zinc is a trace metal and essential nutrient that is ubiquitous in nature and can be toxic at high concentrations. It is present in the soil, air, water, and the biosphere. Its environmental cycling occurs through transport in natural media (e.g. water and air)
and through living organisms (e.g. plant roots and animals). Zinc has been used by humans since antiquity and is one of the most commonly used metals to date in industrial products. While zinc mining thrived in the 20th century, for the most part in North America and Europe [30], the adverse effects of mining activities for the environment and water quality caused several mining camps to shut down and subsequently be filled with water. Acid mine drainage that resulted from biogeochemical reactions in combination with anoxic conditions in abandoned mining camps has gained a lot of scientific interest since. In these conditions, zinc sulfide clusters form and are believed to play an important role in the environmental cycle of zinc and other toxic elements that sorb on particulate matter [8].

The chemistry of metals in anoxic systems is often dominated by reactions with reduced sulfur species, such as HS\(^{-}\) and H\(_2\)S. Although sulfide is an intermediate base, its reduced species are soft bases and tend to form strong complexes with soft acids, such as Zn\(^{2+}\), Hg\(^{2+}\), and Ag\(^{+}\) (Table 1.1). Metal sulfide complexes and clusters form in supersaturated waters and play an important role in the environmental cycling of both metals and sulfur, because they serve as building blocks for the formation of mineral structures. Hydrothermal vent systems, sediments of mine drainage, and biofilms of sulfate reducing bacteria are some examples where ZnS is known to precipitate [8, 31, 32]. Metal sulfide clusters can be stabilized by organic matter and persist as nanoparticles that are often mistaken by researchers for dissolved species [33].
Table 1.1: Pearsons classification of hard and soft acids (only part of the list is shown) [27]

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<td>Hard (class A)</td>
<td>H⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Fe³⁺, As³⁺</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Fe²⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Pb²⁺</td>
</tr>
<tr>
<td>Soft (class B)</td>
<td>Cu⁺, Ag⁺, Au⁺, Hg²⁺, Pd²⁺, Cd²⁺</td>
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The environmental relevance of these very small particles is not limited to the cycling of its constituents, but also the cycling of toxic metals, such as Pb, Cd, and As and radioactive materials that may adsorb on their surface (e.g. [34]). Metal sulfide particles that precipitate abiotically in supersaturated waters or through microbial activity, may be stabilized by DOM and remain suspended for extended periods of time. In addition, manufactured ZnS or ZnS-coated quantum dots, which are often comprised of toxic metal constituents, may be released in the environment. The presence of these particles may hinder water quality.

1.3 Metallic silver nanoparticles

The use of nanomaterials in consumer products has been rapidly increasing in the past couple of decades and subsequently concerns have been raised about the potential impact of these materials to the environment. Some of these materials contain toxic metals, such as silver and cadmium, which once released into the environment
could pose a threat for ecosystems and human health (e.g. [16, 35, 36]). Silver, in
particular, due to its antimicrobial activity, is widely used in consumer products
(including, but not limited to health and fitness products, clothing, food, and medical
applications) [17].

The natural cycling of silver occurs mostly through the corrosion of silver loaded
soils into aquifers, but human activity can affect its environmental fate and transport.
Some examples are mining, smelting, coal burning, and the use of silver in industrial
processes [37]. Due to the photosensitive properties of some silver halides, silver has
been systematically used in manufacturing plants of the photographic industry. This
resulted in elevated silver concentrations in the wastewater of the plant which was
typically discharged directly into surface waters or in the wastewater collection and
treatment system. Because Ag(+I) was known to be toxic for a wide range of aquatic
organisms, the potential environmental impact of the industrial use of silver was first
questioned in the late 90’s.

Studies showed that the bioaccumulation of Ag(+I) is low and that it is most
likely to form insoluble AgCl in marine waters, and AgS in surface waters, sediment
pore waters, wastewater treatment plant effluents, and mine sediments and tailings [38-
42]. The formation of insoluble solids with inorganic ligands is a possible explanation for
the low bioaccumulation of silver, but organic ligands and especially thiol containing
ligands may interfere and alter its availability to living organisms [43-46]. It was also
found that up to about a third of sulfide complexed silver in wastewater treatment plant effluent was in the colloidal form (possibly stabilized by NOM) [39]. In oxic waters AgS is likely to be photoreduced to zero-valent silver, a process that can possibly lead to the production of zero-valent silver colloids; however the effect of NOM in the photoreduction of AgS has not been studied [47].

The incorporation of silver in consumer products by the booming industry of nanomaterials will eventually lead to releases in the environment through direct discharge into natural waters, or through wastewater treatment systems (Fig 1.1). In the past few years a large number of scientific research projects focused on the toxicity of silver nanoparticles for a wide range of organisms focusing on discerning effects that are specific for nanoparticle characteristics. Some studies found that toxicity is related with particle size and others that it is related to the release of silver ions (e.g. [48-50]). Several of these studies, used cysteine to bind and “immobilize” ionic silver in an effort to separate toxicity effects related to silver ions or the nanoparticles. Furthermore, other studies showed that silver nanoparticles may undergo physicochemical transformations when exposed to solution conditions such as ionic strength, pH, metal binding ligands, and NOM (e.g. [51-53]). It is clear that transformations such as dissolution, aggregation, and surface reactions play a key role in the mechanism of toxicity and that in order to understand these mechanisms, such transformations need to be thoroughly understood [54, 55].
Toxicity does not only depend on the characteristics of the particles, but also the characteristic of the organism that is studied. Overall, studies that study the physicochemical characterization of the particles in combination with biological effects indicate that a nano-specific effect is involved in the toxicity to prokaryotic organisms such as bacteria and algal cells, but toxicity towards higher eukaryotic organisms such as algae, fish, fleas, and nematodes is mostly related to the release of ionic silver [56-59]. The difference in response of prokaryotic and eukaryotic organisms, could possibly be due to the availability of biological mechanisms that produce metal-binding ligands (typically rich in thiols).

Figure 1.1 Schematic describing silver flows deriving from the use of silver nanomaterials in consumer products, such as plastics and textiles
Taken from Blaser et al [60]
Nanomaterials are typically manufactured with organic coatings that serve to stabilize the suspension during production. Examples of silver nanoparticle coatings are long chain polymers (e.g. polyvinylpyrrolidone and polyethylene glycol), low molecular weight organic acids (e.g. citric and ethylenediaminetetraacetic acid), and complex organic substances (e.g. gum Arabic). These compounds attach on the particles surface through complexation or hydrophobic interactions and induce steric, electrostatic, or electrosteric stabilization. These coatings may control the fate of nanoparticles in the environment and their effect on organisms [58].

1.4 Sulfhydryl containing organic compounds in the environment

The sulfhydryl chemical group (also known as thiol) is a form of reduced sulfur and a major intermediate in the microbial cycling of sulfur. Thiols are formed during the microbial reduction of sulfur and through abiotic reactions of dissolved organic matter with H₂S and elemental sulfur in pore waters [61]. The biological and environmental importance of thiols lies in their reactivity towards metal ions. Thiols participate in several biological systems, where they serve as coenzymes or antioxidants for binding metals. In aquatic systems, they are mostly present in anaerobic waters, such as sediment pore water, where they bind metals and sorb on colloids [61].
The concentration of thiols varies with the type of medium they are dissolved in. In oxic waters, thiols are present as low molecular weight organic compounds in concentrations that are lower compared to anoxic systems, because they tend to oxidize and form disulfide bonds in the presence of oxygen. Anaerobic pore water of sediments contains nano- to micro-molar concentrations of thiols [62]. Under metal stress some plants and organisms actively excrete thiol rich compounds known as phytochelatins or metallothioneins (e.g. [63, 64]) and the rate of excretion is proportional to the level of metal contamination [65]. In biological media, such as intra-cellular fluids and blood, thiol concentrations can reach up to micro mol per gram (hundreds of milli-molars) and milli-molar, respectively [66, 67].

The molecular structure of thiols may also vary. Thiol functional groups are present in natural waters as part of natural organic matter (NOM) and as smaller organic compounds. The concentration of reduced sulfur in NOM is low compared to other functional groups, such as carboxylates and hydroxyls. Total sulfur in NOM isolates is in the range of 0.4 to 1.4% on a mass basis [68] and 6 to 55% of that can be in the reduced form (sulfides or thiols) in humic acids, depending on the site where the NOM was collected and its origin [69, 70]. However, they serve as strong binding sites for soft metals, such as Ag⁺ and Zn²⁺. Equilibrium formation constants are often used as indicators of reactivity. Formation constants of soft metals with reduced sulfur can be more than eight orders of magnitude higher than with carboxyls and amines (Fig. 1.2).
Although thiols are very reactive with soft metals, their role for the biogeochemical cycling of metals in natural waters is often overshadowed by other more abundant functional groups. For example, studies on the dissolution of HgS and precipitation of ZnS in the presence of several NOM isolates showed that the reduced sulfur content of the NOM was poorly correlated with the dissolution and precipitation rates of the metal sulfides [9, 71]. However, it is possible that the metal concentration in
these studies was high enough to quickly saturate reduced sulfur sites in NOM, leaving properties with less metal specificity (e.g. aromaticity and molecular weight) to control the dissolution and precipitation processes. However, under some environmentally relevant conditions (e.g. sediment porewater) thiol content in NOM is expected to be in excess of the metal. In biological media, where low molecular weight thiols are present in milli-molar concentrations, the speciation of metals is expected to be dominated by complexation with sulfhydryl groups.

1.4.1 Cysteine

Cysteine is a low molecular weight thiol and an aminoacid. It’s an important building block of several proteins and participates in several biochemical reactions, including its contribution to zinc fingers, an important configuration for protein, RNA, and DNA functions (e.g. [72]). In most cases cysteine is part of larger structures, such as proteins and phytochelatins. It is also part of other low molecular weight thiols, such as glutathione a major antioxidant, γ-glutamylcysteine a typical monomer of phytochelatins, and N-acetyl cysteine another antioxidant (Fig. 1.3). Because of its ability to bind metals, cysteine has been used as a model thiol ligand in studies of particle-bacteria interactions, biochemical reactions, and toxicity effects (e.g. [73-75]). In this work, cysteine is used as an example of low molecular weight thiols. Serine, a structurally similar to cysteine amino-acid has a hydroxyl group in the place of the thiol
group in cysteine (Fig. 1.3). The similarity of the two compounds allows for comparing the thiol group of cysteine with the hydroxyl group of serine.

![Molecular structures](image)

Figure 1.3 The molecular structure of cysteine, serine, N-acetyl cysteine, glutathione, and γ-glutamylcysteine

1.5 Processes controlling metal particle formation and persistence in natural waters

DOM often moderates the reactivity and bioavailability of dissolved and particulate matter in natural waters. DOM is involved in many processes that take place during metal cycling in natural waters (Fig. 1.4). The complexity and heterogeneity of the molecules that comprise DOM has lead scientists to categorize different types of DOM based on operational definitions, instead of physicochemical properties. However, in order to understand the mechanisms of interaction between DOM and metallic
constituents it is necessary to study the physicochemical properties of DOM. Ligand groups serve as binding sites for metals and can therefore be used for this purpose. The following sub-sections summarize the processes that involve metal-based nanoparticles in suspension with organic ligands.

![Diagram](image)

**Figure 1.4.** Schematic describing interactions between metals and NOM in natural waters and their effect on bioavailability. Taken from Aiken *et al.* [2]

### 1.5.1 Surface reactions

Surface reactions are physical and chemical phenomena that take place at the solid-water interface between solutes and atoms on the particles surface. Some examples are the formation of chemical bonds, dehydration, surface diffusion, and association.
processes of the adsorbed constituents [76]. Adsorption is an association process that may lead to formation of chemical bonds and is a prerequisite for dissolution and surface chemistry modifications that affect aggregation.

1.5.1.1 Adsorption

Adsorption of solutes on particles can alter surface properties such as charge, surface atom speciation, and morphology that control aggregation, precipitation, and dissolution processes; these processes often determine the fate, transport, and bioavailability of constituents comprising the particles (e.g. [21, 77-79]). Adsorption of a solute on the surface of a solid is controlled by temperature, pressure, concentration of the solute, available surface area, and presence of competing solutes. Solutes such as metal cations, inorganic oxyanions, and organic matter (OM) can adsorb on particles surface. In this work we focus on the latter and specifically organic matter that contains sulphydryl functional groups, due to the high reactivity of these moieties with soft metals such as Ag⁺ and Zn²⁺ (Fig. 1.2). In contrast to cation and oxyanion adsorption, OM adsorption has not been fully understood, due to uncertainties regarding the structure and composition of the complex and diverse organic matter molecules. Adsorption occurs through several mechanisms, including the interaction between specific functional groups in OM and surface constituents (Table 1.2).
Table 1.2: Mechanisms of adsorption for organic compounds in soil solutions [80]

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Principal organic functional groups involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cation exchange</td>
<td>Amines, ring NH, heterocyclic N</td>
</tr>
<tr>
<td>Protonation</td>
<td>Amines, heterocyclic N, carbonyl, carboxylate</td>
</tr>
<tr>
<td>Anion exchange</td>
<td>Carboxylate</td>
</tr>
<tr>
<td>Water bridging</td>
<td>Amino, carboxylate, carbonyl, alcoholic OH</td>
</tr>
<tr>
<td>Cation bridging</td>
<td>Carboxylate, amines, carbonyl, alcoholic OH</td>
</tr>
<tr>
<td>Ligand exchange</td>
<td>Carboxylate</td>
</tr>
<tr>
<td>Hydrogen bonding</td>
<td>Amines, carbonyl, carboxyl, phenylhydroxyl</td>
</tr>
<tr>
<td>Van der Waals interactions</td>
<td>Uncharged, nonpolar organic functional groups</td>
</tr>
</tbody>
</table>

1.5.1.2 Adsorption and the special case of nanoparticles

Adsorption is proportional to the total available surface area of the solid.

Nanoparticles have a greater ability to adsorb solutes compared to their bulk counterparts, due to the high surface area to volume ratio. However, studies have shown that particles with diameter <10 nm exhibit enhanced adsorption capacity even when normalized to the specific surface area (e.g. [81-83]). This “nano-effect” is also true for other processes (e.g. photocatalysis and phase transformations) and for a range of nanomaterials; Auffan et al. argued that these nano-effects should be the basis for defining nanomaterials and suggested that a particle size cutoff of 30 nm is more appropriate for this purpose than the conventional concept according to which a nanomaterial is a structure that has at least one dimension less than the generic size of 100 nm [84]. This definition is important for studies involving nanoparticles, because it distinguishes them from similar studies involving larger colloids.
The enhanced adsorption capacity of nanoparticles can be linked to the total free energy and atomic structure on the surface of particles. Adsorption takes place where it is most energetically favorable, preferentially on sites of structural imperfections, such as steps, kinks, and pits. One cause of structural imperfections that is of particular importance for nanoparticles is the lack of atomic order on the solids surface. While in the center of a crystalline particle, atoms are arranged in space according to their crystal structure, this structure is distorted on the surface because surface atoms only have neighboring atoms towards the center of the particle. This imperfection results in surface relaxation: a slight change on the spacing between the two surface atomic layers that are on the edge of the particle. Sorption of water molecules can enhance this phenomenon [85]. Surface relaxation is especially important for nanoparticles, because a large percent of the total number of atoms in the particle are located on the surface (Figure 1.5).

![Graph showing the relationship between particle size and percent of atoms on the surface.](image)

**Figure 1.5.** The relationship between particle size and percent of atoms on the surface
1.5.1.3 Surface redox reactions

An oxidation-reduction (or redox) reaction is a chemical reaction in which electrons are transferred completely from one species to another [86]. Electrons are used by microorganisms for metabolic reactions. Therefore, redox reactions occur in nature mostly as a result of biological activity, but abiotic redox reactions are also possible. When a ligand adsorbs on a particles surface, outer sphere complexes form. If the ligand has strong affinity for surface atoms (e.g. sulfhydryl functional groups for soft metals), the ligand proceeds to form inner sphere complexes, which can lead to further chemical reactions, including redox reactions.

1.5.1.4 Manufactured nanomaterials with coatings

Natural nanoparticles form under environmental conditions and in the presence of OM that can sorb on their surface and stabilize them. However, manufactured nanomaterials are produced under controlled conditions, often in the presence of a surfactant that sorbs on the particles surface. The surfactants’ primary purpose is to stabilize the particle suspension during production, but they remain on the particles’ surface in the final product and are likely to interfere between the particle and OM in natural waters.

1.5.2 Particle aggregation

Aggregation is the time dependent change of the dispersion state of colloids, towards larger sizes. Because colloid aggregation causes settling and may lead to
separation of the particles from the liquid phase, it is of paramount importance for colloid persistence and the environmental cycle of elements. Therefore, it has been studied extensively, especially in the context of water and wastewater treatment (e.g. [87]).

Settling occurs through the gravitational force applied on a particle and the settling velocity $v_s$ can be estimated from Stokes’ law:

$$v_s = \frac{g \cdot (\rho_s - \rho) \cdot d^2}{18 \cdot \eta}$$

(1.1)

where $g$ is the gravity acceleration, $\rho_s$ the particles’ density, $\rho$ the density of water, $n$ the absolute viscosity of water, and $d$ the particles’ diameter. Assuming spherical geometry and low Reynolds number, the settling velocity of a 100 μm ZnS particle at 20°C is 1.63 cm/s, which means that this particle can fall 5 meters in about 5 minutes. Because the settling velocity is proportional to the square of the particles’ diameter, it would take $10^4$ and $10^8$ times longer for 1 μm and 100 nm particles, respectively to settle over the same distance, which is approximately equal to 35 days and 10 years, respectively. Therefore, particles that do not grow bigger than 100 nm are able to resist settling for a long time and can travel long distances following the flow of water in rivers and lakes. However, in natural aquifers the settling velocity is also influenced by the water chemistry and particle porosity, as well as advection, turbulent diffusion, and dispersion phenomena caused by the movement of water (e.g. [88]). For very small particles (< 100 nm), which is the focus of this work, the effect of water movement on particle stability is
insignificant compared to surface transformations caused by water chemistry, hence they were not studied further.

Colloids are present in all natural waters in a variety of sizes and are often characterized by a continuous particle size distribution. A suspension is stable when its average size distribution remains constant over time, i.e. the particles move about in the water without settling or aggregating. The mechanism of stability for a colloidal suspension depends on particle-particle and particle-organic molecule interactions. These interactions occur as a consequence of attractive and repulsive forces and depend on several factors such as the type and concentration of ions in solution, the presence of organic and inorganic ligands, or NOM, the particles’ shape and size distribution, and the total number of particles.

Higher total number of particles leads to higher chances for collision and hence less stable suspensions. Although the shape and size distribution may vary greatly within a suspension, for simplicity most theoretical models that deal with the interactions between particles assume spherical shape and monodisperse suspensions; an assumption that is oversimplifying actual conditions. Even if the assumption of spherical shape holds, as particles attach on each other, they produce non-uniform clumps of irregular shapes. Hence, the size distribution becomes more complicated. The spherical shape assumption may be valid for the very first steps of aggregation, when
only monomers are present, but the overall size and fractal dimensions of those clumps will affect the following aggregation steps (e.g. [89, 90]).

Interactions between particles also depend on the adsorption of long or bulky organics and the charge on the particles’ surface. Long chain organic polymers and bulky NOM molecules can sorb on particles and cause steric hindrances that stabilize particles. This mechanism is called steric stabilization. On the other hand, a suspension of charged particles is stabilized by electrostatic repulsion and this mechanism is called electrostatic stabilization. Electrostatic forces may result from the adsorption of charged molecules or from the consistency of the particle. When both steric and electrostatic phenomena occur, the stabilization mechanism is called electrosteric.

The presence of surface charge on mineral particles is the result of one of the following processes: isomorphic substitution within crystal structure (metal ions in solution replacing ions on the mineral surface), structural imperfections (caused by co-precipitation of more than one metals), preferential adsorption of specific ions (ions in solution adsorbing on the particle surface on specific sites), and ionization of inorganic groups on particulate surfaces [87]. Surface charge attracts ions of the opposite charge, which form a layer around the surface, known as the Helmholtz layer (or Stern layer) and can be up to 5 Å thick (Figure 1.6). The ions in that layer attract a cloud of ions, of both the opposite and same charge, that surrounds the particle more loosely and forms the diffuse layer. These two layers comprise the electrical double layer (EDL) of a
charged particle, which can extent up to 300 Å. The EDL is characterized by an electronic potential that produces repulsive electrostatic forces between particles of the same charge and is a stabilizing factor. On the other hand, particles are attracted by Van der Waals forces, who are destabilizing factors. Particle stability depends on the balance between these two forces.

Figure 1.6: Electrical double layer of a spherical particle with negative surface charge. Taken from Crittenden et al [87]
The EDL is dependent on the type and concentration of ions in solution. High concentration of ions allows them to counterbalance the electronic potential of the EDL. This causes the EDL to compress and the electrostatic repulsive forces between particles to weaken. As a result, particles aggregate and the suspension is destabilized. The dependence of particle stability with the concentration of ions in solution is typically expressed through the critical coagulation concentration (ccc). As the concentration of ions in solution increases, the surface charge is gradually neutralized and aggregation becomes faster, until the electrostatic forces are much weaker than Van der Waals attractive forces and aggregation occurs as fast as Brownian motion allows. The lowest concentration of ions where this occurs is defined as the ccc. When there are no repulsive forces and aggregation is driven by Brownian motion, aggregation occurs in the diffusion limited regime; otherwise in the reaction limited regime.

The most widely used theory for modeling the repulsive and attractive forces between two charged surfaces was developed by the combined work of Derjaguin, Landau, Verwey and Overbeek, and is known as the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (e.g. [91, 92]). This theory only takes into account the repulsive electrostatic forces and attractive Van der Waals forces. Other forces, such as acid-base interactions and Born repulsion have been included in the form of the extended DLVO theory, which is also a simplified model because it does not account for factors often
occurring in natural systems, such as steric stabilization and particle bridging by polymers [93].

One key feature of the DLVO theory is the Hamaker constant used to calculate the attractive forces between particles, because it is characteristic of the particle surface composition [94]. This constant can be calculated from experimental measurements of a given system and then compared to Hamaker values for certain types of materials from the literature. Comparing the Hamaker constant to published values is used as a criterion for the validity of the DLVO theory for that specific system (e.g. [95]). In cases where organics are adsorbed on the surface of colloids, the DLVO theory often does not apply.

The ccc value and zeta potential measurements are needed to calculate the Hamaker constant of colloids, using the following approximation based on DLVO theory [96]:

\[
ccc \left( \frac{mol}{L} \right) = \frac{(4\pi \cdot \varepsilon_0)^{3/2} \cdot 0.107 \cdot \varepsilon_r^3 \cdot (k_B \cdot T)^{1/2} \cdot Z^4}{N_A \cdot A^2 \cdot (z \cdot e)^2}
\]

(1.2)

where \( A \) is the Hamaker constant, \( \varepsilon_0 \) is the permittivity in vacuum, \( \varepsilon_r \) is the permittivity of water relative to vacuum, \( k_B \) is the Boltzman constant, \( e \) is the electron charge, \( N_A \) is the Avogadro number, and \( z \) is the valence of the electrolyte. The function \( Z \) is defined as

\[
Z = \tanh \left( \frac{z \cdot e \cdot \Psi_0}{4 \cdot k_B \cdot T} \right)
\]

(1.3)
where $\Psi_0$ is the particle surface charge and often assumed to be equal to zeta potential. Aggregation experiments are necessary to calculate the ccc. The change of average hydrodynamic radius over time at the time when aggregation begins is proportional to the initial number of particles [97, 98]:

$$
\left( \frac{dR_H}{dt} \right)_{t=0} \propto k n_0
$$

(1.4)

where $R_H$ is the average hydrodynamic radius, $n_0$ the initial number of particles, and $k$ is the aggregation rate constant. The reaction limited and diffusion limited regimes exhibit different aggregation rate constants and their ratio is called attachment efficiency, $\alpha$, which is the percent of collisions that lead to attachment and is used as an indicator of the suspensions’ tendency to aggregate:

$$
\alpha = \frac{k_d}{k_{d,\text{fast}}}
$$

(1.5)

Hence, for two systems with the same initial total number of particles, attachment efficiency is calculated by dividing the initial rate of change of the hydrodynamic radius in the reaction limited regime with the rate of change in the diffusion limited regime. The latter is independent of electrolyte concentration. Plotting the attachment efficiency over a range of electrolyte concentrations the ccc is calculated by fitting the following equation to the data [97]:

$$
\alpha = \frac{1}{1 + \left( \frac{\text{ccc}}{M^{\text{crit}}} \right)^\beta}
$$

(1.6)
where $M^{n+}$ is the electrolyte ($n = 1$ for monovalent electrolytes) and $\beta'$ is the slope of the data (plotted as log [$\alpha$] versus log [electrolyte concentration]) for $\alpha$ values less than 1.

A large amount of research in the field of colloidal stability has focused on metal oxides, such as iron, manganese, and aluminum oxides. However, less attention has been given to metal sulfides, especially the ones involving trace metals, such as zinc and mercury. In addition, most studies incorporate NOM as one of the independent variables affecting aggregation. However, NOM is not a fully characterized molecule and is thus treated as a “black box”. In order to study the effect of specific properties of NOM (e.g. functional groups, molecular weight, and aromaticity) molecules with a defined structure need to be used.

**1.5.3 Precipitation: growth and aggregation**

Precipitation is the process during which two or more elements in solution, e.g. a metal and a ligand, react to form a solid phase. The importance of this process for the environmental cycle and bioavailability of metals lies in the formation of a separate phase that has the potential to grow and fall out of solution and may be less bioavailable than the soluble forms of the metal. Precipitation occurs in supersaturated solutions, where metals react with ligands to form polynuclear complexes (nucleation) and grow with the further addition of subunits from solution (growth). If the resulting polymers become large enough a new phase is formed, a solid particle (Figure 1.7) [99].
Precipitation is dependent on parameters such as the concentrations of dissolved constituents, pH, temperature, the liquid medium in which precipitation occurs, and coprecipitation. These parameters control the formation of polymers that grow or combine to form the solid phase [100]. Several ligands can be involved in the precipitation of metals; hydroxides, sulfides, carbonates, and phosphates are some typical inorganic ligands. Hydroxide and sulfide, in particular, have been linked to the evolution and adaptation of living organisms during the Earths lifetime because they moderate metal bioavailability [101, 102]. Although metal oxides have been extensively studied, less is known about metal sulfides, especially sulfides of trace metals, such as zinc and mercury. In their classic study, Rickard and Luther gave a detailed summary of what is currently known about the formation and stability constants of metal sulfide complexes and clusters which serve as building blocks for mineral formation [33].
The presence of organic ligands and NOM can decrease the precipitation kinetics of metal sulfides; if they remain small in size these particles are able to persist in natural waters for extended times. Historically, the study of early-stage formation of precipitates has been a difficult task due to the physical state and size of these precipitates. Analytical tools used for dissolved species cannot be used, because the precipitates are in the solid phase, while at the same time they are so small that detection with tools used in colloid science is difficult. With recent advancements in techniques such as small angle X-ray scattering, X-ray absorption spectroscopy, atomic force microscopy, and
electron microscopy, several technical problems have been tackled and we are now able to study particle formation from dissolved species in greater detail.

1.5.4 Particle dissolution

Dissolution is a weathering process during which protons or ligands in solution adsorb on the surface of a solid and form inner sphere complexes with surface atoms. The bonds near the site of adsorption are gradually polarized and chemical reactions may take place. Finally, the surface atom is removed from the solid phase into solution [86]. Parameters affecting dissolution include the concentration of the ligand (e.g. H⁺), the concentration of the dissolving constituent in solution (e.g. dissolved metal), temperature, and the structure of the solid phase. Dissolution is accelerated with increasing ligand concentration and temperature and decreasing concentration of the dissolving constituent and crystalline structure.

Particle size, shape, and aggregation state may also affect the dissolution kinetics of nanoparticulate suspensions. Liu et al found that the surface area normalized dissolution rate of galena nanoparticles was higher than for bulk galena [103]. In addition, they found that certain lattice faces dissolve faster than others and that dissolution of closely packed particles was slower than non-aggregated particles. These findings indicate that dissolution of nanoparticles has some unique characteristics compared to the bulk material.
1.6 Research objectives

The goal of this research was to develop a better understanding of the complexation between metal-based nanoparticles with soft metal constituents and low molecular weight thiols in natural waters, and its impact on the fate of the particles. To achieve this goal, this research was divided into three objectives. The first and second objectives investigate the interactions between cysteine, zinc, and sulfide. The first objective (Chapter 2) was to resolve discrepancies in the current literature and determine whether cysteine can reduce or enhance the aggregation kinetics of ZnS nanoparticles. To achieve this objective, bare ZnS nanoparticles with average monomer size of 10 nm were synthesized. This research aimed at mimicking the precipitation of ZnS nanoparticles in the presence of extracellular biomolecules with thiol constituents. The aggregation behavior of the ZnS nanoparticles was monitored using dynamic light scattering. In combination with zeta potential measurements and adsorption experiments, the mechanism of stabilization was determined.

The second objective (Chapter 3) was to study the effect of cysteine on the early-stage formation of ZnS nanoclusters (less than 10 nm) in supersaturated solutions. Previous studies indicated that thiol containing organic compounds reduce the precipitation and aggregation kinetics of metal sulfides. This research aimed at studying the effect of cysteine on the two distinctive and parallel processes that take place during precipitation: growth and aggregation. A suite of complementary methods was utilized
to study the kinetics of growth and aggregation of ZnS nanoclusters in the presence of excess, equimolar, or lesser cysteine.

The final objective (Chapter 4) was to use similar approaches as in the previous two objectives to study a wider range of transformations that metallic silver nanoparticles may undergo in anaerobic environments. The effect of cysteine on dissolution, aggregation, and surface modifications of metallic silver nanoparticles was studied. The potential role of coatings in these processes was investigated by utilizing particles synthesized with two different coatings that are often used in silver nanoparticle manufacturing.

Finally, in Chapter 5, the implications of this research for understanding the environmental fate of naturally-occurring and manufactured metal-based nanoparticles are discussed.
Chapter 2. Influence of amino acids cysteine and serine on aggregation kinetics of zinc and mercury sulfide colloids

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2.1 Introduction

Colloidal and nanoparticulate metal sulfides play an important role in the speciation of metal pollutants in the anaerobic aquatic environment, and consequently, can control their transport and bioavailability to organisms. Moreover, nanoscale particles (typically smaller than 30 nm), can exhibit size-dependent properties such as increased solubility and sorption capacity (even when normalized to surface area) [81, 103]. Zinc, mercury and other metals exist as mineral sulfide colloids and nanoparticles in environments such as marine ecosystems near hydrothermal vents, sediments of mine drainage, porewater of anaerobic sediments, and treated municipal wastewater effluents [12, 15, 31, 81, 104-106]. Aggregates of ZnS nanoparticles have also been observed as products of biomineralization in the biofilms of sulfate-reducing bacteria in stream sediments [32]. Nanoparticles and colloids of HgS persist in soils and sediments near abandoned mercury mines [10, 107]. These particles are small enough to pass through conventional filters (0.2 or 0.45 μm pore size) and can be mistaken as dissolved species.
in contaminated waters. Furthermore, information regarding the persistence of incidental or naturally-occurring metal sulfide nanoparticles is needed to assess the potential environmental hazards of engineered nanomaterials, such as ZnS-coated quantum dots.

Natural organic matter (NOM) is known to play a vital role in the fate and transport of colloids in the aquatic environment (e.g. [10, 21, 97]). However, little work has been done to identify the NOM functional groups responsible for surface interactions with metal sulfide particles under environmental conditions. In our previous research on ZnS and HgS precipitation [28, 29], we showed that coprecipitation with thiol-containing natural organic acids such as cysteine decreased the precipitation and growth rates of Zn- and Hg-sulfide nanoparticles. While aggregation appeared to be the key process controlling particle growth in these precipitation studies, aggregation could not be separated from nucleation and crystal growth processes that were occurring simultaneously in the particle suspensions. Moreau et al. [104] also studied the effects of cysteine on ZnS colloid aggregation. These authors observed a completely opposite result in that cysteine appeared to enhance colloid aggregation, rather than prevent it. Differences in water and surface composition may be the reason for this discrepancy; however, these parameters were not studied in great detail.

The objective of this work was to investigate the aggregation kinetics of ZnS and HgS colloids and understand how sorption of thiols and water chemistry (e.g., pH, ionic
strength) modifies colloidal surfaces and particle attachment efficiencies. We compared two amino acids, cysteine and serine, which are present in nanomolar to micromolar concentrations in sediment porewater and the anoxic layer of stratified surface waters [62, 108]. These amino acids were chosen because they are structurally analogous except for substitution of the thiol group (on the cysteine) for a hydroxyl group (on the serine). Aggregation experiments were performed to compare how particle attachment efficiencies varied as a function of adsorbed amino acid concentration and water composition (pH and monovalent electrolyte concentration).

2.2. Materials and methods

2.2.1 Materials

All chemicals used in this work were ACS reagent grade and purchased from Sigma-Aldrich unless stated otherwise. Barnstead Nanopure-grade water (>17.8 MΩ cm) was used to prepare all reagents and samples. Trace-metal grade acids were used to adjust the pH of solutions. Borosilicate glass containers for reagents were acid cleaned by an overnight soak in 1 N HCl followed by three rinses with Nanopure water.

A dissolved Zn(II) stock solution was prepared with ZnNO₃•6H₂O (Fisher). Sulfide stock solutions were prepared by dissolving Na₂S•9H₂O crystals (rinsed and dried prior to weighing) in water purged with N₂ (ultrahigh purity). Stock solutions of 1.5 mM L-cysteine and 3.5 mM serine were prepared and stored at 4°C. The cysteine stock solution was utilized within 2 weeks of preparation.
Reagents for cysteine quantification included 2,2′-dithiobis(5-nitropyridine) (DTNP) dissolved in acetonitrile and 0.5 M sodium acetate buffer dissolved in water and adjusted to pH 6. Reagents for serine quantification included 2-mercaptoethanol, o-phthalaldehyde (OPA) dissolved in ethanol, and 0.4 M sodium borate dissolved in water and adjusted to pH 9.5. HPLC-grade solvents were utilized for all reagents.

2.2.2 Synthesis and characterization of ZnS and HgS colloids

A stable suspension of uncoated ZnS colloids was prepared using a method modified from Sooklal et al. [109]. In summary, the sodium sulfide stock solution was added to a zinc nitrate solution, yielding equimolar concentrations of 0.2 mM zinc and sulfide at pH ~10.7. After stirring the zinc sulfide solution for less than 1 minute, the mixture was stored in a capped polypropylene centrifuge tube and allowed to age for at least 4 days at room temperature before use in aggregation experiments. HgS colloids were synthesized using a similar approach, except that the pH of the stock suspension was pH 4.

After the aging period the ZnS stocks contained sphalerite particles with average hydrodynamic diameter equal to 65 nm, according to dynamic light scattering (DLS) (Malvern Zetasizer) and X-ray powder diffraction (Appendix A, Figures A1 and A2). Images collected by transmission electron microscopy (TEM) indicated that the colloids ranged from 40 to 150 nm in diameter (Appendix A, Figure A3). Moreover, TEM images showed that the colloids consisted of aggregates of monomers that were approximately
10 nm diameter. Zeta potential of the particles was -36.6 ± 2.8 mV, which is in agreement with previous work [110]. The BET specific surface area of the particles (freeze dried from aqueous suspension) was 104 m²/g.

The stock HgS suspension consisted of particles with average hydrodynamic diameter near 70 nm. The HgS colloids consisted mainly of cinnabar and/or metacinnabar (Appendix A, Figure A4).

2.2.3 Aggregation experiments

Experiments with the ZnS colloids were performed to determine the effects of pH, ionic strength, and amino acid concentration on aggregation rates and the attachment efficiency, $\alpha$, of the particles. ZnS aggregation experiments were initiated by diluting an aliquot of the ZnS colloid stock suspension in Nanopure water containing NaNO₃ (between 0.075 M and 0.5 M) and buffered from pH 6.5 to 8.5 by 5 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES). The concentration of particles in all experimental samples corresponded to 50 μM (as Zn and S). A selection of the solutions also contained either cysteine or serine, ranging from 5 to 100 μM in concentration. In HgS aggregation experiments, the colloids were diluted to final concentration of 2 μM HgS in HEPES-buffered NaNO₃ solutions.

The samples were mixed in polypropylene centrifuge tubes and immediately transferred to a 1-cm polycarbonate cuvette before analysis by DLS. A selection of mixtures, particularly those of the bare particles (no amino acid) and those with serine,
was mixed directly in the cuvette. The intensity-weighted average hydrodynamic
diameter \(a_h\) of the colloids was quantified over time by DLS using incident light (\(\lambda=633\)
nm) scattered at 173°. Early stage doublet formation rates were approximated by the
slope of the linear least squares regression of data plotted as \(a_h\) versus time [111, 112]:

\[
\left( \frac{da_h(t)}{dt} \right)_{t\to0} = k_{11} \cdot N_o
\]  
(2.1)

where \(k_{11}\) is the aggregation rate constant and \(N_o\) is the initial particle number
concentration. Early stage aggregation was defined for \(a_h\) values equal to or less than
two times the initial \(a_h\). Aggregation experiments were replicated 2-9 times, as
determined by a relative standard deviation of less than 25% for replicate initial growth
rates \(\left( \frac{da_h(t)}{dt} \right)_{t\to0}\).

The attachment efficiency \(\alpha\) was calculated from the aggregation rate constant
normalized by the aggregation rate constant \((k_{11})_{fast}\) in the diffusion-limited regime:

\[
\alpha = \frac{k_{11}}{(k_{11})_{fast}} = \frac{1}{N_o} \cdot \left( \frac{da_h(t)}{dt} \right)_{t\to0,fast} 
\]  
(2.2)

2.2.4 Zeta potential of ZnS colloids

Zeta potential of ZnS colloids was calculated from the electrophoretic mobility
measured at 25°C (Malvern Zetasizer). The samples consisted of ZnS particles
(concentration corresponding to 50 μM ZnS) suspended in 5 mM HEPES buffer and 0.05 M NaNO₃. A selection of samples contained either cysteine or serine. The ZnS colloids were suspended in solution and allowed to aggregate for approximately 30 min prior to electrophoretic mobility measurements. Adjustments of pH were made by addition of HCl or NaOH to the particle suspensions. Electrophoretic mobility was measured in triplicate sample aliquots.

2.2.5 Adsorption of amino acids on ZnS colloids

Adsorption of cysteine and serine on ZnS colloids was quantified to investigate how amino acid sorption might affect colloid aggregation. Batch experiments were prepared by suspending ZnS particles (50 μM) in solution containing 5 mM HEPES buffer, 50 mM NaNO₃, and either cysteine or serine under ambient (i.e., oxic) laboratory conditions. The particle suspensions were mixed end-over-end for 1 h. This adsorption time was chosen because most aggregation experiments were performed within this time frame, and sorbed cysteine concentration was relatively constant over 1 h (Appendix A, Figure A5a). Furthermore, we chose the short sorption time to avoid loss of cysteine by oxidation, which would be more than 10% over ~1 day (Appendix A, Figure A5b). In a 1-h period we observed less than 2% loss of the cysteine in oxic conditions at pH 7.5.

After mixing, the suspensions were filtered with 0.025-μm filters (VSWP Millipore). Zinc concentration in the filtrate was quantified by inductively coupled
plasma atomic emission spectroscopy (Prism Teledyne Leeman Labs). The zinc that remained in the filtered water was 3.5% (±0.5%, n=3) of the initial total Zn, indicating that this filtration method was capable of separating the particles from the aqueous phase. Cysteine concentration was measured in the filtrate by derivatization with DTNP and quantification by reverse phase high performance liquid chromatography (Varian ProStar) [108, 113]. Serine was measured with a method modified from Lindroth and Mopper [114]. Samples were derivatized by addition of 1-mL OPA reagent to 1-mL sample. The serine-OPA derivative was quantified by UV absorbance at 335 nm (Cary-Bio-100 spectrophotometer).

2.3. Results and Discussion

2.3.1 Effects of amino acids, monovalent electrolyte and pH on metal sulfide aggregation

In the ZnS and HgS aggregation experiments, the presence of cysteine in solution decreased aggregation rates relative to controls with no amino acids. Serine did not alter aggregation rates. Attachment efficiencies $\alpha$ were calculated from the time-resolved DLS data for the diffusion-limited and reaction limited growth regimes (e.g., Appendix A, Figure A6). For the range of electrolyte concentration utilized in this study (0.001 - 0.5 M NaNO₃), diffusion-limited ZnS aggregation ($\alpha \approx 1$) was observed for particle suspensions with 100 μM serine and in suspensions with no amino acid added (Figure 2.1a). In the
presence of cysteine (35 and 100 μM), α values were less than 1 over a range of 0.0025-0.1 M NaNO₃.

The critical coagulation concentration (ccc), defined as the NaNO₃ concentration at the threshold between reaction-limited and diffusion-limited aggregation, was calculated by fitting the data to the equation [98]:

\[
\alpha = \frac{1}{1 + \left( \frac{ccc}{[NaNO₃]} \right)^{\beta'}}
\]

where \( \beta' \) is the slope of the data (plotted as log \( \alpha \) versus log [NaNO₃]) for α values less than 1.

The results indicated that the ccc for ZnS aggregation increased as cysteine concentration increased. The ccc values were 0.044 and 0.11 M for suspensions with 35 and 100 μM cysteine, respectively. These data indicated that cysteine enhanced the stability of ZnS colloids.
Figure 2.1. Attachment efficiency, $\alpha$ for aggregation of (a) ZnS colloids (50 $\mu$M as Zn and S) and (b) HgS colloids (2 $\mu$M as Hg and S) suspended in solution with cysteine, serine, or no amino acid (pH 7.5, 5 mM HEPES buffer).

In similar experiments with HgS colloids, the results also demonstrate that cysteine reduced HgS aggregation rates (Figure 2.1b). Serine had no observable effect on aggregation rates compared to controls with no amino acid added. In the presence of 100...
μM cysteine, the ccc of HgS colloidal aggregation was 0.44 M. This value is approximately 0.34 M greater than the ccc values for the amino acid-free control (0.092 M) and the sample with 100 μM serine (0.095 M). For the HgS concentration utilized in the aggregation experiments (2 μM HgS), reaction-limited aggregation regimes were observed in the amino acid-free controls. Reaction-limited aggregation was not observed with the zinc sulfide suspensions containing 50 μM ZnS.

In addition to NaNO₃ concentration, pH also changed the aggregation rates of ZnS colloids suspended in water with cysteine. For water with pH values ranging from 6.5 to 8.5, greater colloidal stability was observed at higher pH values (Figure 2.2). The ccc was 0.035 M at pH 7.5 and approximately 0.5 M at pH 8.5. At a fixed ionic strength (e.g., 0.05 M NaNO₃ in Figure 2.3), pH had a profound effect on the aggregation rate of ZnS colloids. Attachment efficiencies decreased by four orders of magnitude as the pH increased from pH 6.5 to pH 8 (Figure 2.3).
Figure 2.2. Attachment efficiency for aggregation of ZnS colloids (50 μM) in the presence of cysteine and as a function of monovalent electrolyte concentration and pH (35 μM cysteine, 5 mM HEPES buffer).

Figure 2.3. Attachment efficiency for aggregation of ZnS colloids (50 μM) in solution containing cysteine and varying pH (5 mM HEPES buffer, 0.05 M NaNO₃ electrolyte). Error bars represent one standard deviation of 2-9 replicate samples.
2.3.2 Zeta potential of ZnS colloids

Zeta potential of the ZnS colloids was measured as a function of pH for suspensions containing bare particles (i.e., no amino acid) and for ZnS suspensions containing amino acids. Our data in Figure 2.4 indicated that the isoelectric point (i.e.p.) of bare particles was between pH 7 and 8, which is in agreement with some previous work [115-117], but inconsistent with other studies [118-120] that reported i.e.p. values less than pH 3. The discrepancy of i.e.p. values in the literature has been attributed to oxidation of surface S atoms under oxic conditions [119]. Partial dissolution of ZnS particles may also be occurring, particularly at pH values less than 6 [110]. The Zn:S ratio used to synthesize ZnS particles could also change the surface charge of the particles. For example, an excess of HS⁻ ions could sorb to particle surfaces, leading to net decrease in surface charge.

The addition of 100 µM serine did not change the zeta potential of the colloids, but the addition of cysteine decreased zeta potential and i.e.p. values of the particles. Modification of surface charge to more negative values provides an explanation to the stabilization effect of cysteine for the ZnS colloids. As cysteine concentration increased from 35 to 100 µM, large changes in zeta potential were not observed (for pH > 7) even though ZnS aggregation rates decreased under these conditions (Figure 2.3).
Figure 2.4. Zeta potential as a function of pH for ZnS colloids (50μM) suspended in solution with cysteine, serine, or no amino acid (5 mM HEPES buffer and 0.05 M NaNO₃ electrolyte). Error bars represent one standard deviation of 6-9 replicate samples.

The critical coagulation concentration and zeta potential measurements were used to calculate the Hamaker constant for ZnS colloids, using the following approximation based on Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [96]:

$$ c_{cc} \left( \frac{mol}{L} \right) = \frac{\left( 4 \pi \varepsilon_0 \right)^3 \cdot 0.107 \cdot \varepsilon_r \cdot 3 \left( k_B T \right)^{1/2} Z^4}{N_A \cdot A^2 \cdot (z \cdot e)^6} $$

(2.4)

where \( A \) is the Hamaker constant, \( \varepsilon_0 \) is the permittivity in vacuum, \( \varepsilon_r \) is the permittivity of water relative to vacuum, \( k_B \) is the Boltzmann constant, \( e \) is the electron charge, \( N_A \) is the Avogadro number, \( z \) is the valence of the electrolyte. The function \( Z \) is defined as

$$ Z = \tanh \left( \frac{z \cdot e \cdot \Psi_0}{4 \cdot k_B \cdot T} \right) $$

(2.5)
where $\Psi_0$ is the particle surface charge (here assumed to be equal to the zeta potential).

For the ZnS colloidal suspensions at pH 7.5, Hamaker constants were estimated to be $4.4 \times 10^{-20}$ and $2.8 \times 10^{-20}$ J in solutions containing 35 and 100 μM cysteine, respectively. At pH 8.5 and 35 μM cysteine, the calculated Hamaker constant was $1.3 \times 10^{-20}$ J. These values for $A$ are approximately an order of magnitude smaller than other previous reports (generally $A > 10^{-19}$ J) for copper and other metal sulfide particles in water media [121]. Adsorption of cysteine may have changed the surface properties of the particles; however, further studies are needed to evaluate the mechanism of cysteine sorption and the implications for van der Waals interactions between metal sulfide surfaces.

### 2.3.3 Adsorption of cysteine on ZnS

Adsorption of cysteine and serine on ZnS colloids was quantified to understand how the amino acids were interacting with particle surfaces. For the range of cysteine concentrations utilized in the aggregation experiments, cysteine was observed to adsorb to the particles within 1 h (Figure 2.5a). The adsorption isotherms, however, did not differ significantly between pH 6.5 and pH 7.5, even though $\alpha$ values changed by orders of magnitude with 100 μM cysteine in the suspensions (Figure 2.3). Thus, the decrease in zeta potential as the pH shifted from 6.5 to 7.5 (Figure 2.4) was likely caused by deprotonation of surface functional groups (on the mineral surface or adsorbed cysteine molecules) rather than a change in cysteine surface density.
While pH controlled the extent of surface deprotonation and zeta potential, cysteine surface density also greatly affected ZnS aggregation rates and $\alpha$ values. As shown in Figure 2.5b, the attachment efficiency $\alpha$ decreased by three orders of magnitude as cysteine surface density increased from 0.1 to 0.5 $\mu$mol/m$^2$.

We did not observe significant sorption of serine on ZnS colloids at pH 7.5. For dissolved serine concentrations ranging from 50 to 150 $\mu$M, we recovered more than 93.5% of the total serine in the aqueous phase after 1 h exposure to ZnS colloids. Thus, the low binding affinity of serine on the metal sulfide particles is an explanation for no effect on ZnS and HgS aggregation.
Figure 2.5. (a) Sorption of cysteine after 1 h exposure to ZnS colloids (50 μM) at pH 6.5 and 7.5 and (b) effects of cysteine surface density on the attachment efficiency $\alpha$ for ZnS aggregation (pH 7.5, 5 mM HEPES buffer, 0.05 M NaNO$_3$ electrolyte). Error bars represent one standard deviation of 2-9 replicate samples (where available).
2.4 Conclusions

Colloidal and nanoparticulate metal sulfides can govern the speciation of toxic metals such as zinc and mercury in anaerobic environments. Surface coatings by small molecular weight organic acids and humic substances are important for the fate and transport of these particles in sediment porewater and in the water column. Thiol-containing organics are present in natural waters in small concentrations (typically micromolar or less), but have very high affinity for type B metal ions such as Hg$^{2+}$ and Zn$^{2+}$ [27]. Hence, organic molecules with thiol functional groups may preferentially adsorb on mineral sulfides and change particle surfaces in a way that can drastically alter colloidal stability.

Cysteine decreased the aggregation rates of ZnS particles by altering electrostatic interactions between the particles, as indicated by negative surface charge induced by the presence of cysteine. Sorption of cysteine also appeared to change particle-particle van der Waals interactions because the estimated Hamaker constants were lower than values expected for metal sulfides. The results also demonstrated that water composition (e.g., ionic strength and pH) was a parameter that influenced surface charge and aggregation rates of ZnS colloids. Differences in water chemistry and surface charge could be the reason for discrepancies between our previous work [29] and the study by Moreau et al. [104], where pH values and particle zeta potentials were not reported.
This study highlights the importance of thiol-containing organic ligands for metal sulfide colloid aggregation due to relatively high binding affinities between thiols and metal sulfide surfaces. Such interactions may not occur to the same extent with other types of colloids (e.g., silica and other metal oxides). Other thiol-containing organics (including proteins with cysteine components) may enhance metal sulfide particle aggregation rather than prevent it, depending on the size, structure, and hydrophobicity of those organic compounds. Moreover, non-specific processes such as hydrophobic interactions may be important for sorption of humic substances and other NOM macromolecules. Further studies should address both specific and non-specific surface interactions with humic substances and the implications of these interactions for colloidal stability of mineral sulfides.
Chapter 3. Early-stage precipitation kinetics of zinc sulfide nanoclusters forming in the presence of cysteine


3.1 Introduction

Nanoparticles are ubiquitous in the environment and play vital roles in earth processes such as the global cycling of elements, bioavailability of nutrients, and contaminant transport [29]. Nanoparticulate metal sulfides such as ZnS are important for the speciation of metal pollutants in anaerobic and sulfidic settings, and consequently, can control their transport and bioavailability to organisms [122]. Moreover, nanoscale particles (typically smaller than 30 nm) exhibit size-dependent properties such as increased solubility and sorption capacity (even when normalized to surface area) [81, 83, 103].

ZnS and other mineral sulfide nanoparticles tend to exist in organic-rich environments such as anaerobic sediments and municipal wastewater [8, 12, 15, 106, 123-125]. While natural organic matter (NOM) is capable of changing the interfacial chemistry of polynuclear ZnS clusters and nanoparticles and altering precipitation and aggregation kinetics, the mechanism of this process remains poorly elucidated. Such information would provide insight to the conditions required for the formation of metal sulfide nanoparticles in the environment. Furthermore, an understanding of the
processes that enable nanoparticles to persist will aid risk assessments of synthetic nanomaterials (e.g., ZnS quantum dots) that may be released to the environment.

NOM is thought to interfere with the early stages of mineral precipitation by sorbing to the surface of the initial nanocluster or nanoparticle subunits, blocking surface sites from further crystal growth, and modifying interfacial chemistry of the particles that ultimately reduce aggregation and deposition rates [126-128]. This process has been studied extensively for iron (hydr)oxides and other oxide minerals [21, 97, 129-132]; however, little is known regarding such processes for metal sulfides.

Metal sulfides such as ZnS differ from oxide minerals in that thiol-containing moieties associated with NOM may preferentially sorb to metal atoms on the surface of these materials [133] and act as capping agents for the nanoclusters and nanoparticles formed during the early stages of precipitation. ZnS nanoparticles have been observed in the biofilms of anaerobic sediment bacteria [134]. These nanoparticles were closely associated with sulfur-containing organics within the extracellular matrix of the biofilms [104]. Previous research has demonstrated that thiol-containing organic acids such as cysteine, mercaptoacetate, and glutathione were capable of stabilizing nanoparticulate ZnS, CdS, and HgS as they precipitated in supersaturated solutions [28, 29, 135]. The thiols were shown to induce a net negative charge on the particle surfaces, resulting in electrostatic repulsive forces that were unfavorable for aggregation [136].
The objective in this study was to investigate how cysteine altered growth rates of the initial polynuclear zinc sulfide cluster subunits (termed nanoclusters in this study) formed during the beginning stages of ZnS precipitation. We used a combination of small angle X-ray scattering (SAXS) and dynamic light scattering (DLS) to probe changes to the clusters as they grow and aggregate in aqueous suspension. The scattering experiments were complemented with Zn K-edge X-ray absorption spectroscopy (XAS) to determine the speciation of ZnS nanoclusters that formed in the presence of cysteine. Nanocluster products were characterized by additional methods including X-ray diffraction and electron microscopy.

3.2 Materials and methods

3.2.1 Materials

All chemicals used in this work were ACS reagent grade and purchased from Fisher Scientific unless stated otherwise. Barnstead Nanopure-grade water (>18 MΩ cm) was used to prepare all reagents and samples. Ultrahigh purity gases were used for purging solutions. Trace-metal grade HCl was used to adjust the pH of solutions. Borosilicate glass containers for reagents were acid-cleaned by an overnight soak in 1 N HCl followed by three rinses with Nanopure water. A zinc(II) stock solution was prepared by dissolving ZnNO₃•6H₂O crystals in water. Sulfide stock solutions were prepared by dissolving Na₂S•9H₂O crystals (rinsed and dried prior to weighing) in
water purged with N₂. The sulfide stock solution was utilized within 36 hours of preparation. Stock solutions of L-cysteine hydrochloride (Sigma-Aldrich) were prepared in water purged with N₂ and stored at 4 °C. The cysteine stock solution was utilized within 2 weeks of preparation. Sodium bicarbonate solutions were prepared by dissolving NaHCO₃ in water, stored at 4°C and utilized within three days of preparation.

3.2.2 Dynamic light scattering and small angle X-ray scattering

Experimental solutions for dynamic light scattering (DLS) and small angle X-ray scattering (SAXS) consisted of cysteine dissolved in NaHCO₃ (20 or 35 mM) buffered at pH 7.9 to 8.3. ZnS precipitation was initiated by equimolar addition of Zn(II) followed by S(−II) from their respective stock solutions. Zn and S concentrations in the samples were 2 or 5 mM. Various initial concentrations of cysteine were used to determine its effect on the precipitation kinetics of ZnS at room temperature.

The hydrodynamic diameter of Zn-S-cysteine mixtures was measured with time-resolved DLS (Malvern Zetasizer NS) at 25°C. After the addition of sulfide, the sample was dispensed into a 1-cm polycarbonate cuvette and placed immediately into the instrument sample holder for measurement. The intensity-weighted average hydrodynamic diameter of the precipitates was quantified over time by DLS using incident light (λ = 633 nm) scattered at 173°.
Nanocluster size and fractal dimensions of ZnS particles were measured by SAXS at the ID02 high brilliance beamline at the European Synchrotron Radiation Facility (Grenoble, France). The beamline was configured to wavelength $\lambda=0.1$ nm and fitted with a flow-through and stop-flow sampling assembly to allow for time-resolved data acquisition. Scattering data was acquired for 0.01 s or 0.03 s intervals for scattering vector $q$ between $7.5 \times 10^{-3}$ Å$^{-1}$ and 0.4 Å$^{-1}$. Additional SAXS experiments were performed at the BM02 beamline at ESRF, with data acquisition intervals of 100 s for $q$ values between $1.8 \times 10^{-4}$ Å$^{-1}$ and 0.17 Å$^{-1}$. At BM02, sample suspensions were dispensed in quartz capillary tubes (1.5 or 2.0 mm inner diameter, Mark-Röhrchen, Ltd) for analysis. Each ZnS sample had a corresponding blank that involved the same buffer solution (without Zn or S) for which SAXS spectra was collected in the same holder prior to the respective ZnS sample. SAXS data for the samples and the respective blanks were collected for the same acquisition time. Raw data from SAXS experiments underwent standard treatment [137], in which time- and absorption-weighted background scattering of blank samples were subtracted from sample spectra and the corresponding "dark count" of the detector was taken into account for background correction.

### 3.2.3 Zn K-edge X-ray absorption spectroscopy (XAS)

Samples for XAS were prepared by adding aliquots of zinc nitrate and sodium sulfide stocks to a final concentration of 5 mM into solutions containing cysteine. The pH was then adjusted by adding NaOH or HCl. The samples were mixed end-over-end
for 1 h and then centrifuged for 180 min at 11,000 g, 25°C (Sorvall, Super T21). The settled particles were separated from the supernatant and freeze dried for analysis.

Reference materials for XAS included commercially-obtained sphalerite ZnS (Sigma Aldrich), wurtzite ZnS (SPI), and zincite ZnO (Sigma Aldrich) powders. Additional Zn-cysteine references were synthesized by dissolving Zn(NO₃)₂ (5 mM) in a solution containing dissolved cysteine (5 mM or 10 mM), resulting in solutions of Zn(cys) and Zn(cys)₂ complexes. After adjusting to pH 8 with NaOH, the solutions were freeze-dried.

The geometry and local atomic structure of Zn(II) was measured with X-ray absorption spectroscopy (XAS) in the extended fine structure (EXAFS) range. XAS is a powerful tool for structural characterization of nanometer-scaled materials because the measurements have high selectivity between elements and are independent of the long-range order of the material. XAS spectra were recorded at room temperature at beamline X23A2 at the National Synchrotron Light Source (NSLS, New York, USA). The samples were diluted into BN and pressed into thin pellets to achieve an edge jump of approximately unity. Spectra were acquired in transmission mode using a Si(311) monochromator from 100 to 800 eV above the Zn K-edge (9.659 keV). The spectra were compiled from the merge of three to five scans, and the energy was calibrated using a Zn foil. EXAFS spectra were obtained after performing standard procedures for pre-edge subtraction, normalization, polynomial removal, and wave vector conversion using the IFEFFIT software package [138]. For each atomic shell, the interatomic distance (R),
coordination number (CN), and mean squared displacement (ss²) were adjusted and the number of independent parameters was never exceeded during modeling. The amplitude reduction factor S₀² and the threshold position E₀ were fit to data from reference compounds (wurtzite, sphalerite, and zincite) and fixed for all subsequent analyses.

3.2.4 Other particle characterization methods

Sample characterization was also performed with X-ray diffraction (XRD) (Philips X’Pert PRO MRD HR). Aliquots of dried samples (same as samples for XAS) were placed on double-sided tape. XRD spectra were collected with incident beam wavelength of 1.54056 Å. Spectra from the blank tape was used for background subtraction.

Particle morphology was assessed by transmission electron microscopy (TEM) (Hitachi HF2000) operated at 200 keV beam energy. Samples for TEM were prepared by depositing a 10 μl droplet on a formvar-coated copper grid (Ted Pella) and wicking excess liquid with a lint-free tissue. The sample was left to dry for 15 min, and the deposition process was repeated three times. The grid was rinsed by repeating the deposition/wicking step with Nanopure water. Grids were dried in a desiccator for at least 24 h before analysis with TEM.
3.3 Results and discussion

3.3.1 Cluster growth kinetics of ZnS-cysteine suspensions

The addition of dissolved Na₂S to solutions containing dissolved Zn resulted in particles precipitating from solution. Time-resolved SAXS was used to probe growth kinetics of the initial nanocluster subunits with and without cysteine in solution. Log-log scattering plots (intensity $I$ versus scattering vector $q$) did not reveal oscillations or correlation peaks, suggesting that the samples consisted of polydisperse suspensions of particles (Figure 3.1). Scattering intensities were strongly dependent on Zn and S concentration (Figure 3.1a). The scattering intensity at $q$ values near $10^{-2}$ Å$^{-1}$ decreased as ZnS concentration decreased from 5 mM to 0.005 mM (Figure 3.1a), in agreement with previous work [139]. For our experimental conditions, ZnS concentrations in the millimolar range were needed to obtain sufficient scattering intensities. Thus, 2 mM to 5 mM ZnS was chosen for precipitation experiments with cysteine in solution.

For mixtures where cysteine concentration was less than or equal to ZnS concentration (cys:ZnS ratios of 1:4, 1:2, and 1:1), scattering intensity curves had a negative slope at low $q$ values ($q \sim 10^{-2}$ Å), indicating that the particles were aggregating (Figure 3.1b). In contrast, for the sample with excess cysteine relative to ZnS (2:1 ratio), the scattering curve at low $q$ values (from $10^{-2}$ to $10^{-15}$ Å) was flat, indicating little to no aggregation.
Figure 3.1. Small angle X-ray scattering curves of precipitating ZnS particles. (a) Precipitation with varying concentrations of ZnS (no cysteine). (b) ZnS precipitation with cysteine. Sample mixtures consisted of the following: 0.5 mM cys + 2 mM ZnS (1:4), 1 mM cys + 2 mM ZnS (1:2), 5 mM cys + 5 mM ZnS (1:1), 10 mM cys + 5 mM ZnS (2:1). All samples were prepared in 30 mM bicarbonate buffer, pH 8.

DLS data were consistent with aggregation in the samples with low cysteine (1:4, 1:2 and 1:1 cys:ZnS ratios) and no aggregation in the sample with excess cysteine (2:1 ratio). Hydrodynamic diameters of the aggregates were between 100 and 300 nm (Figure 3.2a), for the low cys:ZnS ratios. In contrast, the mixture with excess cysteine relative to ZnS indicated average hydrodynamic diameter of 8 nm by DLS (Figure 3.2a). The rapid decrease of aggregation rates for an excess of cysteine (relative to Zn and S) was in agreement with previously reported results [29].
Figure 3.2. (a) Average hydrodynamic diameter of cysteine-ZnS cluster aggregates determined by dynamic light scattering; (b) Fractal dimension quantified from small angle X-ray scattering data (calculated from \( q \) range \( 10^{−1.7} \) to \( 10^{−0.7} \) Å\(^{-1} \)). Sample mixtures consisted of the following: 0.5 mM cys + 2 mM ZnS (1:4), 1 mM cys + 2 mM ZnS (1:2), 5 mM cys + 5 mM ZnS (1:1), 10 mM cys + 5 mM ZnS (2:1). All samples prepared in 30 mM bicarbonate buffer, pH 8.

The SAXS data also provided information regarding the size and growth rates of the aggregate subunits (i.e., the nanoclusters) in the ZnS-cysteine samples. With increasing concentration of cysteine (from 1:4 to 1:2 cys:ZnS ratios in Figure 2.1b), the SAXS curves demonstrated larger intensity at the high \( q \) range (>\( 10^{−1} \) Å\(^{-1} \)), indicating larger subunits or denser clusters [140].

While the SAXS data indicated polydisperse size distribution of particles, the precise nature and structure of these particles were not known. Indeed we were not able to derive the amount of cysteine bound to ZnS and the exact structure of the ZnS-cysteine nanoclusters from our present data. Therefore, rigorous modeling of the SAXS data based on atomic scattering factors and interatomic distances was not possible in this case. However, the sizes of different sub-structures within the aggregates were...
estimated for the 1:1 and 2:1 cys:ZnS mixtures by modeling portions of the experimental data with simple geometric shapes. TEM images of the samples suggested that the monomer subunits were globular shapes (Figure 3.3). Therefore, we utilized the simplest shape, the sphere, for the SAXS models.

![Figure 3.3. TEM images of ZnS-cysteine precipitates. (a) 5 mM cys + 5 mM ZnS (1:1); (b) 10 mM cys + 5 mM ZnS (2:1).](image)

Rayleigh’s equation was used to compute the models for the SAXS intensity $I(q)$:

$$I(q) = N^2 (3 \frac{\sin qR - qR \cos qR}{(qR)^3})^2$$  \hspace{1cm} (3.1)
where $R$ is the radius and $V$ is the volume of the sphere. Standard least squares were used to fit the models to the experimental data (Figure 3.4a and 3.4b).

In the 1:1 cys:ZnS sample at the 0.5 and 3 h time points, the absence of a Porod region at the highest $q$ values suggested that the smallest subunit in this polydisperse suspension of ZnS particles was smaller than the detection limit (< 0.8 nm). The scattering at 13 h of aging displayed a Porod region (indicated by slope of log $I$ versus log $q$ approaching -4). The SAXS data (for $q > 10^{-1}$ Å$^{-1}$) was fit with a model for spherical particles with 2.2 nm diameter (Figure 3.4a). The negative slope of the intensity curve at $q$ values from $10^{-2}$ to $10^{-1}$ Å$^{-1}$ indicated that these 2.2 nm units have minimal spacing between each other or are in contact. These results suggested that the nucleated subunits were growing over the first several hours of the experiment (Figure 3.4c).
Figure 3.4. Small angle X-ray scattering data of 5 mM ZnS precipitating with (a) 5 mM cysteine (1:1 cys:ZnS) and (b) 10 mM cysteine (2:1 cys:ZnS). Solid lines denote experimental data; dotted lines denote Rayleigh model for monodisperse spherical particles. (c) ZnS cluster diameter estimated from Rayleigh models in parts (a) and (b). Solid symbols (●) and (■) correspond to 2:1 and 1:1 cys:ZnS samples, respectively. Open symbol (○) corresponds to diameters for the 2:1 cys:ZnS determined from Guinier plots. Minimum ZnS cluster size at 0.5 h and 3 h time points for the 1:1 cys:ZnS sample were less than the detection window corresponding to the maximum $q$ value ($10^{-3.3}$ Å) for SAXS data collection.

Similarly in the 2:1 cys:ZnS sample, cluster sizes were observed to increase with time. At this cys:ZnS ratio, none of the scattering profiles displayed Porod behavior, indicating again that the smallest structural unit within the aggregates was a ZnS-based cluster smaller than 0.8 nm. The remarkable feature was the absence of aggregates for
the 1 and 3 h time points as shown by the flat scattering curve towards low $q$. The best fits for the low $q$ portion of the scattering curves were obtained with sphere models of 3.2 and 3.9 nm diameter for 1 and 3 h, respectively. These maximum sizes were in close agreement with radii of gyration $R_g$ determined by the Gunier approach [137, 142], thus validating the spherical shape used in our Rayleigh modeling approach. At 13 h of aging, aggregation occurred. The "S" shape scattering profile was indicative of the aggregation of non-touching sub-structures with diameter of 5.7 nm, based on modeling of the intermediate $q$ range of the SAXS data. The spacing between the sub-structures was presumably due to the presence of excess cysteine. These results were in agreement with the TEM observations (Fig. 3.3b) Overall, the SAXS data indicated that an increase in cysteine concentration resulted in faster nanocluster growth rates, while at the same time the cysteine prevented or slowed aggregation of the monomer clusters. With cysteine concentration that was double the ZnS concentration, thiol sorption at the surface was likely sufficient to decrease aggregation rates but insufficient for blocking cluster growth sites. We did not test the effects of increasing cysteine concentration beyond the 2:1 cys:ZnS ratio; however, previous work with CdS clusters suggest that the size of cluster subunits would decrease if cysteine concentration increased further by one or two orders of magnitude relative to the metal sulfide concentration [135].
3.3.2 Structure of aggregates

The DLS and SAXS data both indicated that the 1:4, 1:2, and 1:1 cysteine:ZnS mixtures contained aggregated nanoclusters. The DLS results also indicated that hydrodynamic diameters of the aggregates were relatively constant over the first 13 h of precipitation (Figure 3.2a). Fractal dimension $D_f$ of the aggregates was calculated from the SAXS scattering intensities using the following equation:

$$I(q) \sim q^{D_f}$$  \hspace{1cm} (3.2)

For portions of the log-log scattering curves that were linear ($q$ values ranging from $10^{-1.7}$ to $10^{0.7} \text{Å}^{-1}$), $D_f$ values were estimated from the slope of the log-log SAXS data (Figure 3.2b). In the case of the 1:1 cysteine:ZnS sample, the fractal dimension during the first 30 min was approximately 1.1, indicating linear aggregate structures. After 3 and 13 h, $D_f$ values increased to 1.6, approaching structures that resembled diffusion controlled aggregation kinetics ($D_f \approx 1.7$). At lower cysteine to zinc ratios (1:4 and 1:2), $D_f$ values were approximately 2 for all time points measured except a smaller value at the first time point (0.5 s) (inset, Figure 3.2b). These results indicated that at lower cysteine concentrations (1:4 and 1:2 ratios) aggregation was occurring under the reaction limited kinetics, even though previous work suggested that overall aggregation rates decreased with decreasing cysteine coverage on ZnS particles [136]. Furthermore, aggregation of the initial nucleated clusters was occurring on a faster time scale than we were able to observe (less than 0.5 s). While fractal dimension is an indicator of aggregation kinetics
(i.e. diffusion limited or reaction limited), the nanocluster subunits were also growing and possibly leading to changes in aggregate structure.

### 3.3.3 Particle characterization and speciation

XRD was used to confirm long-range atomic structure (e.g., crystal lattice) of the particles while Zn K-edge XAS was used to determine short-range order (e.g., Zn coordination environment). The diffraction patterns of the ZnS nanoclusters with and without cysteine (Figure 3.5) demonstrated peaks at 28.5°, 47.9°, and 56.5° (2θ units) and are in agreement with the crystallographic d-spacing of the reference compound sphalerite (cubic ZnS). Those peaks correspond to the (111), (202), and (311) lattice planes, respectively. In the nanoparticulate ZnS (n-ZnS) and cys-ZnS (2:1) samples, the broad profiles and the large width at half maximum of the peaks highlight the small size of the crystallites. Using the Debye-Scherrer formula [143, 144], we estimated coherent diameters of 3.5 (±0.4) nm, 3.8 (±0.4) nm, and 3.6 (±0.4) nm for the (111), (202), and (311) lattice planes of the n-ZnS particles (no cysteine). This calculation indicated that the clusters were relatively isotropic, in agreement with the globular shape observed by TEM. For the cysteine-capped ZnS samples, the broad peak at 28.3° corresponded to a diameter of 1.8 (±0.2) nm. In this sample, we observed additional peaks at 29.3° and 31.5°, coinciding with the peaks for the Zn(cys): reference sample. These peaks probably corresponded to residual salts such as NaNO₃ (29.38°, 31.9°) and NaCl (31.7°) that precipitated from solution during the freeze-drying process.
Figure 3.5. X-ray diffraction spectra of the ZnS-cysteine coprecipitated nanoparticles (5 mM ZnS, 10 mM cys), ZnS nanoparticles (no cysteine), ZnS\(_{\text{(a)}}\) and ZnO\(_{\text{(a)}}\) commercial materials, and freeze-dried Zn(cys) and Zn(cys)\(_2\) complexes. The dotted vertical lines correspond to positions of the major peaks for sphalerite-like structures in the ZnS-cys samples. The peaks denoted by * are probably caused by excess salts (e.g. NaNO\(_3\), NaCl) produced during sample preparation.

We used X-ray absorption spectroscopy to study the short-range order and size of ZnS nanoclusters. Experimental Zn K-edge EXAFS data and fits for the n-ZnS and the cys-ZnS samples are shown in Figure 3.6, and the modeling results are summarized in Table 3.1. For all ZnS-cysteine samples, the interatomic distance (R = 2.34 ± 0.02 Å) and coordination number of the first coordination sphere (3.7 ± 0.7 < CN\(_{\text{Zn-S1}}\) < 4.1 ± 0.8) were consistent with Zn atoms tetrahedrally coordinated with S atoms. The bond length was not modified with respect to the bulk sphalerite or wurtzite (R = 2.34 ± 0.02 Å) (Table
3.1). The addition of other atomic contributions for the first coordination sphere (e.g. oxygen) did not significantly improve the fit quality. This result indicated that inner sphere coordination between surface Zn atoms and sulfur atoms (either sulfide or thiol) were dominant in the first coordination sphere for all ZnS samples, with or without cysteine. In contrast, the EXAFS spectra for the Zn-cysteine reference compound indicated a mixture of bonds with coordination numbers of 1.6 ± 0.3 for Zn-O bonds (R = 2.07 ± 0.02 Å) and CN of 2.0 ± 0.4 for Zn-S bonds (R = 2.29 ± 0.02). These results are in agreement with the dissolved Zn-cysteine complexes observed by others [145].

For the atoms within the Zn second coordination sphere of the cys-ZnS samples, coordination numbers (CNZn-Zn and CNZn-S2) and interatomic distances varied with respect to bulk ZnS and were sensitive to cysteine concentration. In the case without cysteine, the second sphere CN values for Zn-Zn (R = 3.85 ± 0.02 Å) and Zn-S2 (R = 4.52 ± 0.02 Å) bonds were 3 to 4 times lower than the bulk sphalerite (Table 3.1). In the EXAFS simulations for the n-ZnS cluster samples (with and without cysteine), we defined CNZn-S2 to equal CNZn-Zn, based on the sphalerite-type structure suggested by XRD spectra (Figure 3.5). For the cys-ZnS sample spectra, we attempted to include second sphere Zn-C coordination, as shown in Table 3.1 for the Zn-cysteine reference compound (CNZn-C = 1.9 ± 0.4 with R = 3.05 ± 0.02 Å). However, the incorporation of Zn-C did not improve the fit quality. This result indicated that most of the S atoms in the first atomic shell were sulfide-S and not thiol-S.
Figure 3.6. Experimental and fitted Zn K-edge X-ray absorption spectra. (a) \( k^3 \)-weighted EXAFS spectra; (b) magnitude of the Fourier transform, including fit to first and second atomic shells. (♦) experimental data, (—) fit.

The \( \text{CN}_{\text{Zn-Zn}} \) values within the second atomic shell represent the average over all Zn atoms in a polynuclear compound with short-range coherent structure. This average \( \text{CN}_{\text{Zn-Zn}} \) can be used to determine the size of the ZnS cluster. Indeed, the overall decrease in \( \text{CN}_{\text{Zn-Zn}} \) values highlights the small size of the n-ZnS cluster, in which a large fraction of Zn atoms were ‘surface’ atoms. We theoretically recalculated the \( \text{CN}_{\text{Zn-Zn}} \) for different size of ZnS clusters (Figure 3.7). While the mean \( \text{CN}_{\text{Zn-Zn}} \) for ‘infinite’ sphalerite particles is 12, we estimated that a 1 nm-sphalerite cluster is characterized by a \( \text{CN}_{\text{Zn-Zn}} \) value close to 5. This estimation assumes that the clusters are isotropic and relatively monodisperse (as indicated by XRD spectra in Figure 3.5 and TEM images in Figure 3.3).
Using the CN$_{Zn-Zn}$ obtained by EXAFS (Table 3.1), we estimated the size of the n-ZnS clusters with and without cysteine (Figure 3.7). We found that a CN$_{Zn-Zn}$ of 3.5 ± 0.7 characterizing the n-ZnS (Table 3.1) might be encountered for a ZnS cluster containing between 5 and 8 Zn atoms with size ranging between 0.8 to 1.2 nm (Figure 3.7). These EXAFS simulations do not take into account the multiple scattering paths of Zn-S-S (11.5% of the amplitude of the Zn-S$_1$ path) and Zn-Zn-S (26.8% of the amplitude of the Zn-S$_1$ path) that are in phase opposition with the simple scattering within the second coordination sphere. Thus, the cluster size obtained in Figure 3.7 might be underestimated, and the ranges in size shown in Figure 3.7 reflect this uncertainty. Moreover, the coherent diameter calculated from XRD data (3.6 ± 0.4 nm) is larger than the one estimated by EXAFS (1 ± 0.2 nm) in agreement with previous work suggesting that XRD measurements in polydisperse systems are weighted towards the larger particle sizes [146]. In addition, XRD is sensitive only to crystallized species, while the EXAFS is sensitive to all the Zn species including those with short-range order. The discrepancy with the EXAFS results suggests that the 3-4 nm sphalerite crystals detected by XRD were in minority.
Table 3.1: Model fitting parameters coordination number (CN), interatomic distance (R), mean squared displacement (ss²), and chi-square residue ($\chi^2$) for Zn K-edge EXAFS data shown in Figure 3.6. Spectra of samples (nano-ZnS and cysteine-capped ZnS nanoclusters) were compared to spectra of sphalerite, wurtzite, zincite, and a freeze-dried Zn(cysteine)$_2$ solution. Fit ranges: $k = 3.9$-$10.5$ Å$^{-1}$; $r = 1.2$-$4.5$ Å. Errors: $\partial$(CN) $\approx 20\%$; $\partial$(R) $\approx 0.02$ Å; $\partial$(ss²) $= 0.001$.

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<th>R</th>
<th>ss²</th>
<th>$\chi^2$</th>
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$^{(a)}$ The Zn-X$_1$ and Zn-X$_2$ shells correspond to atoms present in the first (short distance) and second (long distance) coordination spheres, respectively.
Figure 3.7. Average second sphere Zn-Zn coordination number (CN) calculated as a function of the polynuclear zinc sulfide nanocluster size, based on interatomic distances in the mononuclear ZnS₄ tetrahedra. (♦) denotes theoretical values, (□) denotes CN values from EXAFS data.

For the ZnS samples precipitating in the presence of cysteine, the EXAFS data also indicated lower CN values for the second coordination sphere (Table 3.1) compared to bulk sphalerite; however, the CN values depended on cysteine concentration. In the sample that contained excess cysteine (2:1 cys:ZnS ratio), CN values for Zn-Zn and Zn-S₂ were similar to the n-ZnS formulated without cysteine. A CN_{Zn-Zn} value of 3.5 ± 0.7 (Table 3.1) might correspond to clusters composed of 5 to 8 Zn atoms with a mean diameter about 1 ± 0.2 nm (Figure 3.7). In that case, the size obtained by XRD (1.8 nm) is
closer to estimations by EXAFS, indicating the presence of small sphalerite crystals in this sample.

In contrast, the sample with lower cysteine concentration relative to ZnS exhibited a smaller CN\(_{\text{Zn-Zn}}\) value (1.2 ± 0.24) and CN\(_{\text{Zn-S2}}\) value (1.8 ± 0.36), indicating that the nanoclusters were composed of two ZnSi tetrahedra that were approximately 0.8 nm in diameter (Figure 3.7). Clusters of this size would probably not be observable by XRD (data was not obtained). Overall, the EXAFS results for the cys-ZnS samples were consistent with SAXS data (Figure 3.4), which indicated that nanocluster growth kinetics was faster in the sample with excess cysteine (2:1 cys:Zn).

### 3.4 Conclusions

Our experiments sought to understand how cysteine, a Zn-binding ligand, altered the precipitation and aggregation kinetics of ZnS particles. The early stages of ZnS precipitation resulted in ZnS nanoclusters with structures similar to sphalerite (as shown by XRD and EXAFS data in Figures 3.5 and 3.6). Cysteine altered the rates of nanocluster growth and aggregation by binding to the surface of ZnS particles and changing the composition at the particle-water interface. Our SAXS and EXAFS data also indicated that sorption of cysteine prevented aggregation and facilitated the growth (or crystal ripening) of the zinc sulfide cluster subunits. These results suggested that the cysteine sorption was enough to prevent cluster aggregation, presumably by
electrostatic repulsion [136]. However, surface coverage by sorbed cysteine molecules was not enough to block ZnS growth sites on the cluster subunits. Furthermore, aggregation that occurred at lower cysteine concentrations decreased the driving force for monomer growth or blocked growth sites on the cluster subunits. Additional studies that consider higher cysteine sorption density are needed to determine if cysteine is capable of blocking cluster growth sites, as suggested by previous research with CdS [135].

Our study demonstrated the value of using a combined approach (SAXS, XRD, DLS, and EXAFS) for charactering the ZnS clusters during the initial stages of precipitation and aggregation. Time-resolved SAXS provided information on the size of the cluster subunits without additional modification (i.e., drying) of the particle suspensions. EXAFS, DLS, and XRD allowed for additional information concerning aggregate and cluster size as well as Zn speciation. While TEM provided direct visualization of the size and shape of particles, agglomeration is a possibility during sample preparation. Thus, we used TEM as supporting information for size and morphology. While SAXS methods enabled elucidation of cluster characteristics as they were forming in aqueous suspension, a major drawback of this method was the need for concentrations in the millimolar range. This concentration is relatively high for most environments except for the most contaminated scenarios. Nevertheless, this work
provided clues to the manner in which natural organic ligands influence ZnS cluster as they precipitate under supersaturated conditions.

Overall, our study points to conditions that would enable ZnS nanoparticles to form in the environment and how natural organic acids are critical for enabling their persistence at the nanoscale. In this case, our results show that surface sorption of an organic ligand is required to prevent aggregation of ZnS nanoparticles that nucleate from solution. Furthermore, the sorbed organic ligand allows for the nucleated nanoclusters to increase in size without necessarily aggregating with other particles. We used cysteine as a surrogate for the strong metal-binding ligands (thiols) associated with natural organic matter (NOM) that is ubiquitous in the aquatic environment. NOM, however, consists of complex macromolecular structures that contribute to other surface interactions (such steric hinderances) for aggregation and interfacial chemistry[71]. Further studies should consider the macromolecular properties of NOM and their influence on ZnS nanocluster nucleation and aggregation kinetics.
Chapter 4. Cysteine-induced modifications of zero-valent silver nanomaterials: Implications for particle surface chemistry, aggregation, dissolution, and silver speciation

This chapter was submitted for review at the journal *Environmental Science & Technology*.

### 4.1 Introduction

The wide use of silver nanoparticles (Ag NPs) in commercial products has recently gained much attention due to the potential toxic effects resulting from their unintended release into the environment [16, 147]. The reactivity and transport of Ag NPs in the aquatic environment will depend on key transformation processes, which include aggregation, dissolution, and surface modifications by metal-complexing ligands. These processes work in synergistic or antagonistic ways to influence the lifetime of nanoparticles in the environment and the bioavailability of silver to exposed organisms (Fig. 4.1). For example, adsorption or desorption of organic matter or metal-binding ligands will modify the surface of nanoparticles. Ionic compounds or long-chain moieties can induce surface charge or steric effects that influence particle coagulation and settling kinetics [77, 97, 136]. Aggregation can lead to changes in reaction rates (such as dissolution and sorption) by decreasing the surface area that is exposed to bulk solution [82, 148, 149]. Dissolution of nanoparticles will in turn decrease the size of the
particles and increase dissolved metal concentration in water, which in turn affect ligand adsorption and desorption kinetics.

Figure 4.1. Schematic describing the effect of water chemistry on Ag NP bioavailability. pH, electrolytes, and dissolved organic matter can lead to surface modifications, dissolution, and particle aggregation and affect environmental persistence and the mode of exposure to organisms.

In natural waters Ag(+)I is expected to form strong bonds with ligands such as chloride, sulfide, and other forms of reduced sulfur (e.g. organic thiols) [27]. Ag(+)I is capable of forming relatively insoluble mineral phases (e.g. Ag₂S, AgCl) that can serve as terminal fates for monovalent silver in freshwater, wastewater, and seawater [38, 40, 150]. Recent studies showed that Ag NPs dissolve very slowly in water [151], but
oxidative dissolution is faster in sulfide- and chloride-rich environments [53, 152-154]. In
waters rich with organic matter, sulfhydryl-containing organic compounds (thiols) may
compete with inorganic ligands and increase dissolved silver concentration [43]. Hence,

thiols have the potential to play a key role for the environmental fate of Ag NPs.

In natural waters thiols tend to be associated with low molecular weight organic
ligands and with humic substances. In sediments and water, thiols are typically present
in nanomolar to micromolar concentrations, depending on environmental conditions
such as redox potential, the presence of organisms that actively excrete thiol-containing
compounds, and other metals that may catalyze the oxidation of thiols [62, 63, 155, 156].
In addition, thiol-containing biomolecules are abundant in biological media such as
bacterial plasma, tissues, and blood [66, 67].

Hydrophilic thiols of low molecular weight (such as glutathione,
mercaptoacetate and cysteine) are known to decrease the aggregation and precipitation
rates of metal-sulfide nanoparticles by adsorbing to their surface and changing the
particle surface charge [28, 29, 136]. Cysteine (CYS) has also been used in Ag NP toxicity
studies to chelate dissolved silver ions [57, 157, 158]. Some of these studies noted that
cysteine increased the dissolution of silver and slowed aggregation of Ag NPs; however,
these phenomena were not studied in detail [57, 157]. In order to predict and understand
Ag NP toxicity to organisms, we need to identify the physico-chemical processes that
these particles undergo in the presence of ligands, and particularly thiol-containing ligands such as cysteine [58].

The goal of this study was to better define the effect of low molecular thiols such as cysteine on Ag NP behavior and speciation in aquatic settings. Aqueous suspensions of metallic Ag NPs were exposed to dissolved cysteine, and the dissolution, aggregation, and surface modifications of the nanoparticles were monitored. We compared cysteine reactivity to Ag NPs manufactured with two common types of synthetic coatings used on Ag NPs: citrate and polyvinylpirrolidone (PVP). Cysteine was selected because of this amino acid’s well-defined structure (relative to humic macromolecules) and because cysteine is widely used in Ag NPs toxicity experiments to assess the effects of dissolved Ag⁺. In addition, we used X-ray absorption near edge spectroscopy (XANES) to study the speciation of silver in Ag NPs following exposure to cysteine.

4.2 Materials and Methods

4.2.1 Ag NP synthesis and characterization

Synthesis of zero-valent silver nanoparticles with citrate and PVP (55 kDa molecular weight) coatings (hereafter referred to as Ag-CIT and Ag-PVP, respectively) followed previously published methods [159, 160]. Particle monomer size and shape were characterized with transmission electron microscopy (TEM) (Appendix B, Figure B1). Number-based size distributions of the two nanomaterials were estimated from the
TEM images by counting the number of particles, sorting them into 5-nm or 2-nm size intervals, and plotting the counts in histograms (Appendix B, Figure B1). All samples containing the Ag-CIT nanoparticles were prepared in plastic containers and cuvettes, while Ag-PVP samples were prepared in glass containers (due to loss of PVP-coated nanoparticles on the walls of plastic containers).

Ag-CIT primarily consisted of spherical particles with some oval-shaped particles. The average geometric diameter was 19.1 ± 12.7 nm (n = 188). Ag-PVP consisted of spherical particles with average geometric diameter of 7.6 ± 2.0 nm (n = 106). The specific surface areas (SSA) of the Ag NPs was estimated by summing the surface areas of all particles in each increment in the size distribution (assuming spherical geometry) and dividing the total surface area by the total mass of particles:

$$SSA = \frac{\sum \pi \times d_{i}^2 \times n_{i}}{\rho \times \sum \left( \frac{\pi \times d_{i}^3}{6} \right) \times n_{i}}$$

(4.1)

In Eq. 4.1, $i$ is the bin number in the size distribution histogram (Appendix B, Figure B1), $d_{i}$ is the average diameter in bin $i$, $n_{i}$ is the total number of particles in bin $i$, and $\rho$ the density of silver (10.50 g/ml [161]). We calculated SSA to be 16.1 m$^2$/g for Ag-CIT and 66.1 m$^2$/g for Ag-PVP.
4.2.2 Exposure of Ag NP to cysteine

Stock solutions of approximately 8 mM cysteine were prepared in degassed water, stored at 4°C, and utilized within two weeks of preparation. All nanoparticle dissolution and aggregation experiments were performed with a buffer solution consisting of 7 mM sodium bicarbonate (Fisher Scientific, Pittsburgh, PA) adjusted to pH 7.5, 10 to 500 mM NaNO₃, and 400 μM cysteine. Experiments were initiated by diluting an aliquot of silver nanoparticle stock suspension in the buffer solution. A selection of control solutions included Ag NPs suspended in the bicarbonate buffer without cysteine or with 400 μM serine instead of cysteine. Serine and cysteine are structurally analogous in that serine has a hydroxyl group where cysteine has a thiol group. Particle concentrations in all treatments corresponded to 7.6 to 8 μM total silver.

4.2.3 Quantification of dissolved silver and cysteine

Each mixture of Ag NPs was produced in 6 to 14 replicates. Dissolved silver and cysteine concentrations were measured 20 minutes to 48 hours after preparation. For each time point, two replicates were ‘sacrificed’ for filtration with 0.025 μm membrane filters (VSWP Millipore) fitted on a glass vacuum filtration apparatus. Before filtering the suspensions, the membranes were primed with 10 ml of a solution containing 7.5 mM NaHCO₃ at pH 7.5. This solution was passed through the filter and then discarded prior to filtering sample suspensions. Dissolved silver was nominally defined as the silver concentration in the filtrate, which was quantified with inductively coupled
plasma mass spectroscopy (Agilent Technologies, Santa Clara, CA) after acidifying the filtered samples with 2% HNO₃ and 1% HCl.

The concentration of cysteine in aliquots of the filtered samples was quantified using a previously described method [108, 113] that employed derivatization of CYS, separation with reverse phase high performance liquid chromatography, and detection by UV absorbance.

Control experiments were performed to confirm that dissolved Ag-CYS complexes, Ag⁺, and free CYS were able to pass through the filters. In these experiments, we prepared three control solutions: 1) 9.3 μM AgNO₃ + 400 μM CYS (pH 7.5, 10 mM NaNO₃); 2) 1.9 μM AgNO₃ (pH 8.3); and 3) 100 μM CYS alone (pH 7.3, 10 mM NaNO₃). These mixtures were filtered through the 0.025 μm membrane filters. The recovery of silver in filtrates of the AgNO₃ and AgNO₃+CYS mixtures were 94.1% and 92.1%, respectively. The percentage of total CYS quantified in the filtrate was 92% in the CYS-only mixture and 90% in the AgNO₃+CYS mixture.

The removal efficiency of nanoparticles by the filtration system was tested by quantifying the retention of silver after filtration of Ag NP stock solutions. Less than 0.3% (±0.05) and 11% (±0.5) of the total silver were recovered after filtration from Ag-CIT and Ag-PVP stock suspensions, respectively, indicating that these filters were capturing most of the nanoparticles in suspension. Although the monomer diameters for a portion of the Ag NPs were smaller than the nominal filter pore size, our measurements of
hydrodynamic diameters suggested that the particles were somewhat aggregated in their stock suspensions. Moreover the retention of nanoparticles on the filters could have occurred via hydrophobic or electrostatic interactions between particles and the membrane filter.

4.2.4 Aggregation of NPs in the presence of cysteine

We performed measurements of hydrodynamic diameter to determine if cysteine altered the aggregation rate of Ag NPs. The light intensity-weighted average hydrodynamic diameters in the Ag NP-cysteine mixtures were determined by dynamic light scattering (DLS) (Malvern Zetasizer) using incident light (λ = 633 nm) scattered at 173°. Zeta potential was calculated from the electrophoretic mobility of the silver nanoparticles measured in triplicate at 25°C (Malvern Zetasizer). The average hydrodynamic diameter $a_h$ was monitored over time until it reached twice the initial diameter. In cases where aggregation was too slow, $a_h$ was monitored up to 48 hours. Growth rates, attachment efficiencies, and critical coagulation concentrations (ccc) were calculated using previously described methodologies [98, 162, 163]. In summary, early stage aggregation rates were approximated by the slope of the linear least squares regression of data plotted as $a_h$ versus time;

$$\left(\frac{da_h(t)}{dt}\right)_{t \to 0} = k_{11} \cdot N_o$$  \hspace{1cm} (4.2)
where $k_{11}$ is the aggregation rate constant and $N_o$ is the initial particle number concentration. Aggregation experiments were replicated 2-9 times, as determined by a relative standard deviation less than 25% for replicate measurements of $\left(\frac{da_b(t)}{dt}\right)_{t\to0}$.

The attachment efficiency $\alpha$ was calculated from the aggregation rate constant normalized by the aggregation rate constant in the diffusion limited regime $(k_{11})_{fast}$:

$$
\alpha = \frac{k_{11}}{(k_{11})_{fast}} = \frac{1}{N_o} \frac{\left(\frac{da_b(t)}{dt}\right)_{t\to0}}{\left(\frac{da_b(t)}{dt}\right)_{t\to0,fast}}
$$

(4.3)

### 4.2.5 Silver speciation of Ag NPs

The speciation of silver particles that collected on the membrane filters was assessed using silver L3-edge X-ray absorption near edge spectroscopy (XANES). The Ag L3-edge XANES can be used to study the oxidation state of silver due to a prominent peak exhibited by the spectra at the adsorption edge [164]. For each Ag NP-CYS mixture, approximately 100 ml of sample was passed through the 0.025 μm filters. The filter membrane was stored at -20 °C for one hour, freeze dried, and stored in a desiccator before analysis.

Reference materials for XANES data analysis included PVP- and citrate-coated Ag NPs collected on the membrane filters directly from their stock suspensions. Reference materials for monovalent forms of silver included Ag(+)I-cysteine powders formulated with 1:1 and 1:2 silver:cysteine molar ratios (Ag(CYS) and Ag(CYS)2), Ag-
citrate, and commercially purchased powders of AgNO₃, AgCl, Ag₂O, and Ag₂S. The SI section contains details regarding the preparation of these reference samples.

XANES spectra for the samples were collected at room temperature in fluorescence mode at the Stanford Synchrotron Radiation Lightsource (SSRL) beam-line 4-3. Background subtraction and normalization of spectra were performed using the Athena software package following previous procedures [138]. Linear combination fitting (LCF) for the Ag NP+cysteine samples was performed by fitting a binary mixture of reference spectra in the range 20 eV below and above the adsorption edge (3351 eV). We report the relative proportions and the error was determined by the software’s least squares fitting module. Best fits were considered the ones with the lowest R-factor, $\chi^2$, and $\Delta\chi^2$, which are fitting residual parameters described further in Appendix B.

4.3 Results and discussion

4.3.1 Dissolution of Ag NPs exposed to cysteine

In our batch experiments, the presence of cysteine not only increased the amount of dissolved silver released from Ag-CIT and Ag-PVP nanoparticles, but the cysteine also appeared to modify the surface composition and aggregation rates. In the absence of CYS, the nominally dissolved silver concentration in the diluted Ag-CIT and Ag-PVP suspensions was less than 10% of the total silver. However, in the presence of 400 μM CYS (in Ag NP mixtures containing 7.6 to 8 μM total Ag), dissolved silver increased
Within the first 12 hours and appeared to reach a maximum concentration in 24-48 h (Figure 4.2). At the 48 h time point, the dissolved silver concentration was 36.4 ± 2.2 % of total silver for Ag-CIT and 47 ± 5.6 % for Ag-PVP. When Ag NPs were exposed to serine instead of cysteine, the dissolved silver was similar to the control without any amino acids (Figure 4.2), suggesting that Ag-thiolate interactions were important for Ag dissolution.

![Figure 4.2](image)

**Figure 4.2.** Concentration of nominally dissolved silver (0.025 μm filter) in mixtures of (a) Ag-CIT and (b) Ag-PVP nanoparticles suspended in solutions containing 7 mM NaHCO₃, 10 mM NaNO₃, and either 400 μM cysteine, 400 μM serine, or no amino acid. The solution pH was 7.5 to 8.1. The total silver concentrations measured in unfiltered samples are indicated by dashed lines.

Our results contrast a previous study [165] in which Liu et al. reported that thiols, including CYS, inhibited dissolution of Ag NPs. These contradictory results likely stem from differences in experimental methodology. For example, our control experiments indicated that our method of filtration separate dissolved silver from particulate, while
the centrifugal ultrafilters (utilized by Liu et al.) have been reported to retain significant amounts of dissolved silver [59]. In addition, cysteine is capable of forming polymers/particles with Ag(+) [166], particularly at low ratios of cysteine:silver (e.g. 5 or less) (Appendix B, Figure B2). We did not observe the formation of particulate silver for cysteine: silver molar ratio of 50. Therefore, in this study we formulated our Ag NP-cysteine mixtures with this ratio to prevent the formation of Ag-CYS particles.

From the dissolved silver data in Figure 4.2, we calculated an observed initial dissolution rate by taking data points from the first 12 h and estimating the slope using linear regression. The error for these rates was estimated as one standard deviation of the slope. In the presence of cysteine and 10 mM NaNO₃, the initial dissolution rate of Ag-PVP was 0.15 ± 0.01 µM/h, which was greater than the corresponding dissolution rate for Ag-CIT (0.10 ± 0.01 µM/h) (Table 4.1).

Surface area and particle size can be limiting factors for dissolution of sample constituents [103, 167]. From the specific surface areas of the Ag NPs, we calculated that the surface area-normalized dissolution rate of Ag was 282 ± 25 µmol m⁻² h⁻¹ for the Ag-PVP and 748 ± 96 µmol m⁻² h⁻¹ for the Ag-CIT (both with 10 mM NaNO₃) (Table 4.1). The Ag-PVP dissolution experiment was repeated with 2.0 µM Ag-PVP, which corresponded to the same total surface area (13.9×10⁻³ m² L⁻¹) as the samples with 8 µM Ag-CIT (Table 4.1 and Appendix B, Figure B3). The dissolution rate for Ag-PVP (264 ± 16.9 µmol m⁻² h⁻¹) was still less than for Ag-CIT.
Although the surface area-normalized initial dissolution rate was slower in the case of Ag-PVP, the relative proportion of silver that was released into solution after 48 h was greater in the Ag-PVP mixtures than the Ag-CIT mixtures (47 ± 5.6 % and 36 ± 2.2 %, respectively). One possible explanation for this difference is that the relative ‘accessibility’ of particle surfaces for cysteine molecules was lower for Ag-PVP particles than for Ag-CIT, resulting in slower dissolution rates for the Ag-PVP particles. While we have no direct evidence to demonstrate this phenomenon, we observed a similar indication that reactions at the surface of PVP-coated nanoparticles were relatively slow in aggregation experiments (Appendix B, Figure B4). Here, the aggregation of PVP-coated Ag NPs exhibited an initial lag period ranging from 10 to 50 minutes, during which the hydrodynamic diameters of the particles were constant. After this lag period, the diameters increased linearly with time. No lag period was observed with the citrate-coated nanoparticles.
Table 4.1: Initial dissolution rates of Ag-PVP and Ag-CIT solutions containing 400 μM CYS and 10 mM NaNO₃ (stable suspension) or 100 mM NaNO₃ (aggregating in the diffusion-limited regime). Rates were also normalized to the total surface area of non-aggregated particles in suspension at the initial time point.

<table>
<thead>
<tr>
<th>Type of Ag NP</th>
<th>Tot. Ag (μM)</th>
<th>NaNO₃ (mM)</th>
<th>Total Surf. Area (m² L⁻¹)</th>
<th>Silver dissolution rate (μM h⁻¹)</th>
<th>Surface area-normalized dissolution rate (μmol m² h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag-CIT</td>
<td>8.0</td>
<td>10</td>
<td>13.9×10⁻³</td>
<td>0.10 ± 0.01</td>
<td>748 ± 96</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>100</td>
<td>13.9×10⁻³</td>
<td>0.08 ± 0.01</td>
<td>626 ± 50</td>
</tr>
<tr>
<td>Ag-PVP</td>
<td>7.6</td>
<td>10</td>
<td>54.2×10⁻³</td>
<td>0.15 ± 0.01</td>
<td>282 ± 25</td>
</tr>
<tr>
<td></td>
<td>7.6</td>
<td>100</td>
<td>54.2×10⁻³</td>
<td>0.13 ± 0.01</td>
<td>236 ± 20</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>10</td>
<td>13.9×10⁻³</td>
<td>0.04 ± 0.00</td>
<td>264 ± 17</td>
</tr>
</tbody>
</table>

4.3.2 Aggregation and zeta potential of Ag NPs exposed to cysteine

The presence of cysteine in the Ag NP suspensions not only increased the dissolution rate of Ag from the nanomaterials, but also altered the aggregation rate and electrophoretic mobility of the particles in suspensions (Figures 4.3 and 4.4). In the same Ag NP mixtures corresponding to our dissolved Ag data (Figure 4.2), the Ag-CIT and Ag-PVP nanoparticles aggregated slightly over 48 hours in the absence of CYS (Figures 4.3a and 4.3c, respectively).
Figure 4.3. Average hydrodynamic diameter and electrophoretic mobility of Ag-CIT nanoparticles - (a) and (b) - and Ag-PVP nanoparticles - (c) and (d) -; the nanoparticles (8 and 7.6 μM total Ag, respectively) were suspended in mixtures consisting of 7 mM NaHCO₃, 10 mM NaNO₃, and either 400 μM CYS, or no amino acid. Error bars indicate standard deviations of duplicate experiments for diameter measurements and of triplicate experiments for electrophoretic mobility measurements.

With the addition of CYS, the average hydrodynamic diameter of Ag-CIT at time 0 h was similar to the Ag-CIT suspension without CYS (Figure 4.3a). With time the hydrodynamic diameter of particles in the Ag-CIT+CYS mixtures decreased, potentially due to particle dissolution or disaggregation.
In the Ag-PVP mixtures, the average hydrodynamic diameters at the initial time point decreased from 67.4 nm in the absence of CYS to 46.6 nm in the presence of CYS. The decrease in Ag-PVP diameter due to CYS exposure was potentially caused by dissagregation of aggregates in the nanoparticle stock or perhaps by the replacement of PVP (a long chain polymer with an average molecular weight of 55,000 Da) with CYS (a small amino acid with molecular weight 121 Da) on the surface of the nanoparticles. For the nanoparticles stabilized with citrate (a low molecular weight coating relative to PVP), the influence of CYS on hydrodynamic diameter at the initial time point was not as drastic.

The zeta potential of Ag-CIT particles increased from -37.6 mV to -29.0 mV (at 24 h) in the presence of 400 μM CYS (Figure 4.3b). The net increase in zeta potential could be due to the exchange of the trivalent citrate anion for the monovalent cysteine anion (at pH 7.5) on the surface of the Ag-CIT nanoparticles. In contrast, the zeta potential of Ag-PVP particles decreased in the presence of CYS. The zeta potential was -13.8 mV after 24h in solution without CYS; in the sample with CYS, the zeta potential at 24 h was -38.4 mV (Figure 4.3d). This observation is probably due to the lack of surface charge on original Ag-PVP particles and that the PVP coating mainly provides steric (rather than electrostatic) stabilization for the nanoparticles. For both types of Ag NPs, the zeta potential data indicated that CYS was modifying the surface of the nanoparticles,
potentially by sorbing to the surface and by replacing the initial citrate and PVP coatings.

At a relatively low ionic strength (10 mM NaNO₃), the presence of cysteine had a small effect on aggregation, or perhaps slowed aggregation of the particles in suspension (Figure 4.3). However, at higher NaNO₃ concentrations (up to 1000 mM), aggregation rates increased for both Ag-PVP and Ag-CIT suspensions (Figure 4.4). For example, the Ag-CIT suspensions aggregated faster at ionic strength values between 15 mM and 1,008 mM. The critical coagulation concentrations (ccc) for the Ag-CIT suspensions decreased from 53 ± 5.3 mM in the absence of CYS to 35 ± 2.2 mM in the presence of 400 μM CYS (Figure 4.4a). The decrease in ccc was consistent with electrophoretic mobility measurements showing that CYS induced a net shift towards neutral surface charge for the particles (Figure 4.3b).

The Ag-PVP suspensions (without CYS) did not aggregate appreciably within 48 h in solutions containing a large range of ionic strength values (from 10 mM to 1000 mM NaNO₃). The presence of CYS in the Ag-PVP suspensions caused the particles to aggregate. Based on the attachment efficiencies calculated from the aggregation experiments, we estimated a ccc value of 33 ± 5.3 mM for Ag-PVP particles in the presence of CYS (Figure 4.4b). The observations that CYS induced aggregation (rather than inhibited aggregation) was inconsistent with observed changes in zeta potential (Figure 4.3d). Rather, CYS likely replaced the PVP coating, resulting in loss of steric
repulsive forces enabled by the PVP. The similarity of ccc values between Ag-CIT and Ag-PVP particles exposed to CYS support the assumption that CYS was able to replace the coatings of both suspensions. The differences in absolute aggregation rates could be due to differences in the initial particle number concentration or by differences in rate of exchange of the surface coatings.

Figure 4.4. Attachment efficiency for homo-aggregation of Ag-CIT (a) and Ag-PVP (b) suspensions comprising of 8 and 7.6 μM silver, respectively, 7 mM NaHCO₃, and 400 μM CYS at pH values between 7.5 and 8.1. In some experiments no cysteine was added. Ionic strength was controlled with NaNO₃ at concentrations ranging from 0.01 to 1 M.

4.3.3 The effect of aggregation on dissolution

Aggregation of the nanoparticles is expected to change the amount of available surface area for surface reactions such as dissolution. Thus, we compared how initial dissolution rates changed for suspensions that were relatively stable (10 mM NaNO₃) and suspensions of fast aggregating particles (Appendix B, Figure B5) with an ionic
strength value (100 mM) greater than the ccc for both nanoparticles. For both Ag-CIT and Ag-PVP, the initial dissolution rates of the fast aggregating samples were lower than in stable suspensions (Table 4.1). TEM images of the fast-aggregating samples (collected for Ag-PVP suspensions at the 2 and 24 h time-points, Appendix B, Figure B6) provided evidence for dendritic aggregate structures, which could result in decrease of exposed surface area for dissolution [149].

4.3.4 Speciation of particulate silver

XANES analysis was used to determine the oxidation state and speciation of particulate silver in samples containing Ag NPs and cysteine (Figure 4.5 and Appendix B, Figure B7). Three component linear combination fitting (LCF) of the spectra was performed assuming a binary mixture of the original nanomaterial (Ag-CIT or Ag-PVP) and one of the Ag(+I) reference compounds (Table 4.2 and Appendix B, Tables B1 through B4). LCF attempts using four components did not improve these fits; thus, we do not show these results. The XANES analysis indicated that up to 10% of silver in the particles was oxidized to Ag(+I) and coordinated to reduced sulfur, as compared to the original oxidation state of each respective particle suspension. Fitting with several silver-sulfur references such as Ag₂S, Ag(CYS), and Ag(CYS)₂ yielded very similar values of R-factor, $\chi^2$, and $\Delta\chi^2$, indicating that the nature of the bond between silver and sulfur could be similar to one or a combination of these references. We selected fits with Ag(CYS)₂ as
the representative silver-sulfur model compound for further comparisons between the Ag NP preparations (Table 4.2 and Figure 4.5).

Ag-PVP particles that were exposed to CYS exhibited a higher percentage of oxidized silver (7.2% and 7.9% at 2h and 24 h) than Ag-CIT (3.7% and 6.8%, respectively) (Table 4.2). This observation could be explained by our results that the Ag-PVP particles were smaller than Ag-CIT and would comprise a greater proportion of silver atoms at the particle surface [167]. We note that relative differences of a few percentages in XANES modeling must be interpreted carefully. The relatively smaller errors reported from the fitting software (shown in Table 4.2) do suggest a difference in the amount of oxidized silver for the Ag-CIT and Ag-PVP samples. However, in previous XANES studies that used LCF for known sample mixtures [168], the results of mixing ratios showed that the error from LCF calculations can be up to ±5%.

Overall, our interpretation of the XANES data suggested that a portion of the silver atoms (presumably on the surface of the Ag NPs) were in the monovalent oxidation state and were coordinated to reduced sulfur (either cysteine or sulfide).
Figure 4.5. Silver L3-edge XANES spectra of the original Ag NPs, Ag(+I)-cysteine complexes Ag(CYS)$_2$, and silver nanoparticles that were exposed to cysteine for 2 or 24 h: (a) Ag-CIT; (b) Ag-PVP. The nanoparticles (8.0 or 7.6 μM total silver in Ag-CIT and Ag-PVP samples, respectively) were exposed to 400 μM CYS in mixtures containing 7 mM NaHCO$_3$, 10 mM NaNO$_3$, and pH 7.5 to 8.1. After this exposure period, the particles were collected on filter membranes for XANES analysis. The data points correspond to linear combination fits for spectra of the original Ag NPs and Ag(CYS)$_2$ reference material.

4.3.5 Cysteine adsorption

Cysteine was also quantified in the filtered Ag NP+CYS mixtures and showed that dissolved cysteine decreased within 48 h in both nanoparticle samples (Appendix B, Figure B8a). A larger decrease of dissolved cysteine was observed in the Ag-PVP suspensions than in the Ag-CIT suspensions. This difference was potentially due to the larger specific surface area of the Ag-PVP particles relative to Ag-CIT particles. To test
this hypothesis, we calculated the change in dissolved cysteine concentration (relative to the initial concentration) and normalized these values to the total initial surface area of Ag NPs (Appendix B, Figure B8b). For both types of nanomaterials, the change in dissolved cysteine concentrations were approximately 1 to 2 mmol per m² of NP after 48 h of mixing. Based on the errors (propagated from the CYS measurements), there were no apparent differences between Ag NPs (Appendix B, Figure B8b).

Although the loss of dissolved cysteine could be interpreted as adsorption to on the Ag NPs, the amount of cysteine lost (31 and 96 µM) was greater than the total Ag silver concentration in suspensions (~8 µM). Thus, sorption would not fully explain the loss of dissolved cysteine. Control experiments with cysteine in the buffer solution without Ag NPs were performed to determine if cysteine would oxidize under the experimental conditions. In this experiment, we quantified a loss of approximately 25 µM dissolved cysteine in a 48 h period (Appendix B, Figure B8a). This amount of cysteine oxidation is close to the decrease of dissolved cysteine in the Ag-CIT mixture (31 µM), but did not fully account for the loss of dissolved cysteine (96 µM) in the Ag-PVP mixture. Other reactions at the surface of the PVP-coated Ag NPs, such as the production of reactive oxygen species (e.g. superoxide, hydrogen peroxide, hydroxyl radicals) as the silver nanoparticles oxidize and dissolve, may subsequently react with and oxidize cysteine. While dissolved O₂ appears to be a necessary electron acceptor for oxidative dissolution of silver nanoparticles [153], we have no knowledge of direct
evidence for the production of reactive oxygen species during this process, and further study is needed to understand the reaction products of oxidative dissolution of Ag NPs.

4.4 Conclusions

This study highlights the multiple transformations that can occur when silver nanoparticles are exposed to Ag-binding ligands that can modify the surface composition of the particles and their aggregation and dissolution rates. In our experiments, cysteine increased the solubility of the particles; however, the dissolution rate depended on the aggregation state of the particles and surface modifications caused by sorption of cysteine. All of these processes (surface modifications, aggregation, and dissolution) work in concert to influence the overall persistence and composition of the silver nanomaterials in aqueous suspension. Furthermore, these simultaneous transformations could alter the bioavailability of the nanomaterials to exposed organisms. For example, the presence of Ag⁺-binding ligands are expected to increase the amount of dissolved Ag that can be internalized by aquatic organisms; however, the rate of dissolution would depend on the aggregation state of the particles and surface modifications by the ligand. Furthermore, previous studies have shown that cysteine and other low molecular weight thiols can reduce silver toxicity to organisms exposed to silver nanomaterials [57]. While complexation of dissolved Ag⁺ by cysteine is the likely mode of action (due to the relatively strong affinity between Ag⁺ and cysteine), our
results indicated that cysteine can also induce aggregation of the nanomaterials, leading to a secondary mechanism by which the presence of cysteine could decrease Ag NP toxicity.

The transformations discussed in our study appeared to take place on the surface of the particles, which was originally coated with PVP or citrate. Nanomaterials are typically manufactured with organic coatings that serve to stabilize the suspension during production. In this work we demonstrated that the dissolution rates of PVP-coated particles (when normalized to the specific surface area) were slower than citrate-coated particles. One possible explanation for this phenomenon is that diffusion of cysteine molecules at the particle-water interface of PVP-coated nanoparticles is slower than diffusion at the interfacial region of citrate-coated nanoparticles. Another possible explanation is that citrate is capable of binding Ag⁺, resulting in competition between citrate and cysteine for ligand sorption sites on the nanoparticle surface. Overall our results demonstrate the importance of the coatings for the reactivity of Ag NPs.

Cysteine was used in this study as an example of metal-binding organic ligands that are ubiquitous in the aquatic environment. The fact that serine did not cause similar effects indicated the importance of strong Ag⁺-binding ligands (i.e., thiols) for interactions with Ag NPs. Cysteine can be found in nanomolar concentrations in natural waters [62] (e.g. sediment porewater, surface waters and wastewater) and is also a component of complex organic structures, such as proteins and natural organic matter.
(NOM). In these waters, the concentration of all thiol-containing ligands would be higher than the expected concentrations for Ag NPs. Such observations have been made in previous studies investigating wastewater containing silver contamination derived from the photographic industry [41].

Our study indicated that Ag NPs was altered by cysteine, but further research is needed to demonstrate whether cysteine will have the same effects on Ag NPs when it is part of a more complex structure such as proteins or NOM. Steric effects are expected to occur with macromolecular structures and may alter the rates of reactions between thiolate functional groups and the surface of Ag. Recent studies have indicated that molecular weight and aromaticity of NOM are more important properties than reduced sulfur content in determining the dissolution of HgS cinnabar particles and the precipitation and aggregation of ZnS nanoparticles [9, 71]. Future studies could consider the macromolecular structure of more complex ‘ligands’ such as humic substances, particularly as they influence coupled transformations of the Ag NPs (aggregation, dissolution, surface reactivity) and bioavailability to exposed organisms.
Chapter 5. Conclusions

5.1 Summary

This research studied the complexation between a low molecular weight metal binding ligand and metal-based nanoparticles, focusing on the impact of this interaction for the fate of the particles. The overall goals were to study (1) the growth and aggregation of naturally-occurring metal sulfides and (2) the fate and speciation of metal-based engineered nanoparticles, in thiol rich aquatic systems. Zinc sulfide was used as an example for the first goal and metallic silver for the second. Cysteine was used as a surrogate of low molecular weight thiol ligands.

In Chapters 2 and 3 the effect of cysteine on the growth and aggregation of zinc sulfide were studied using DLS, SAXS, XRD, EXAFS, and chemical analysis. The molar ratio of cysteine to zinc and sulfur determined the kinetics of growth and aggregation in supersaturated solutions. In addition, the mode of stabilization incurred by cysteine was determined: cysteine adsorbed on the surface of zinc sulfide nanoparticles; at pH values higher than 7, deprotonation of cysteine induced electrostatic charge on the surface, thus stabilizing the particle suspension. The fact that serine, a hydroxyl analogue to cysteine did not induce similar effects suggested that thiols have the potential to enhance the persistence of metal sulfides in aquatic systems. These results shed light into the processes that cause nanoparticulate metal sulfides to form and persist in anaerobic waters.
In Chapter 4, the effect of cysteine on the surface chemistry, aggregation, and dissolution of zero valent silver nanoparticles was studied. Cysteine modified the surface chemistry of the silver particles and induced aggregation and dissolution. The extent of surface modifications depended on the coating of the particles. The dissolution rate of particles coated with a polymer, PVP was approximately three times slower than the particles coated with an anion and silver ligand, citrate. These results demonstrate the need to consider multiple and interlinked transformation processes when assessing the bioavailability, environmental risks, and safety of nanoparticles, particularly in the presence of metal-binding ligands.

Overall, the results of this study demonstrated how low molecular weight thiol ligands may control the fate of naturally-occurring and manufactured metal-based nanomaterials. Cysteine sorbed through the thiol group on both zinc sulfide and metallic silver particles, inducing changes on their surface properties. While cysteine stabilized the zinc sulfide, it destabilized the silver particles. The main difference that affected aggregation rates between the two types of suspensions was the coating on silver particles. Manufactured nanomaterials are often produced with surface coatings and our results demonstrated that these coatings may play a critical role in the fate of the materials and need to be incorporated into risk assessment models, bioavailability, and toxicity studies.
5.2 Implications and future research

Nanoparticulate zinc sulfides and other metal sulfides have been detected in hydrothermal vent systems, acid mine drainage, and in the vicinity of sulfate reducing bacteria. The biogeochemistry and bioavailability of these materials should not be expected to be the same as bulk metal sulfide, because nanomaterials are more reactive and may pass through biological membranes, such as cell membranes of bacteria and fish gills. The paradigm for the environmental fate and transport of metal sulfides has been recently revised, after the discovery of metal sulfide nanoparticles that could pass through conventional filters and be mistaken for dissolved. However, the processes that lead to their persistence are poorly understood. Interactions of the metal sulfides with DOM are assumed to be responsible for their stabilization. This research shed light on the interactions of a DOM ligand group with metal sulfides. It was demonstrated that low molecular weight thiols may play a critical role in the fate of naturally-occurring nanoparticles in sulfidic environments, such as anaerobic sediment pore water, biofilms of sulfate reducing bacteria, and municipal wastewater effluent.

Metal sulfide nucleation and biomineralization models may need to be revised. Nanoparticles are intermediates that form during the mineralization of metals and are more soluble than bulk minerals. Hence, the use of thermodynamic data from bulk metal sulfides may not be applicable at the nanoscale [169]. Our results and other studies suggest that strong metal binding ligands, such as thiols may need to be
incorporated in models of metal sulfide precipitation in sediment porewater, biofilms of sulfate reducing bacteria, and other thiol-rich systems [106, 135].

Further work is needed in order to improve our understanding of the biogeochemistry of metal sulfides. One approach to improve the chemical equilibrium model may be to incorporate other metal ions (e.g. iron and copper) in the precipitation of zinc sulfides with thiol ligands. In these scenarios co-precipitation is likely to occur [76]. In addition, thiols bind strongly to Fe$^{2+}$ and Cu$^{2+}$ and are also likely to adsorb on metal sulfide particles. Finally, the presence of a mix of particles, such as metal sulfides and oxides is likely to cause hetero-aggregation, which would increase aggregation kinetics and settling. Another approach may be to incorporate a wider variety of ligands. For example, inorganic ligands (e.g. chloride and ammonia), complex thiol-containing organic ligands (e.g. phytochelatins and metallothioneins), and complex organic matter (e.g. fulvic and humic acids, or extracellular polymeric substances) add to the complexity of the biogeochemistry of metal sulfides.

Knowledge gained from studying the fate and transport of naturally-occurring nanomaterials may be applied for understanding transformations that manufactured nanomaterials may undergo. The same tools may be used for assessing the potential risk of nanomaterials. This study demonstrated that thiol ligand groups are reactive towards both zinc sulfide and metallic silver nanoparticles. Using chemical analysis and DLS we demonstrated that cysteine adsorbed on both types of particles and altered their
aggregation kinetics. XAS was employed to study the speciation of the metals exposed to cysteine. The results of our work suggested that the coating on manufactured nanoparticles may act as a moderating factor for the interactions of manufactured particles with DOM. Based on our findings in Chapter 4, long chain polymeric coatings may be more efficient in protecting the surface of nanomaterials from ligand groups in DOM. Our results suggest that coatings distinguish manufactured from naturally-occurring nanomaterials and should be taken into consideration in models predicting the toxicity, bioavailability, and biogeochemistry of the nanomaterials.

Further research is needed to elucidate the effect of DOM on manufactured nanomaterials. As with the case of naturally-occurring metal sulfides, the presence of (in)organic ligands (e.g. sulfur, chloride, and ethylene diamine tetraacetic acid), complex organics with thiol moieties (e.g. phytochelatins), and complex organic matter (e.g. humic and fulvic acids) are likely to interact with metal-based manufactured nanomaterials. Studies have already shown the ability of inorganic ligands and DOM to react with silver nanoparticles (Chapter 4). Data produced from these studies may be useful for assessing the environmental impact of nanomaterials. A better understanding of the physicochemical properties of nanomaterials and their reactivity in natural waters is sorely needed for the development of risk assessment tools [170, 171]. The findings of this research may be used in such tools, but our results suggest that further work is
needed, especially towards categorizing coatings in respect to their ability to polarize surface atoms, or block reactive sites on nanomaterials.

Finally, the results of this study may be used for designing improved and more environmentally friendly materials. For example, due to their antimicrobial activity, silver nanoparticles have the potential to be used for coating water filtration membranes to prevent the buildup of biofilms [172]. A recent study by Gunawan and collaborators showed that it might be possible to use carbon nanotubes enriched with silver nanoparticles for water treatment with hollow fiber membranes [173]. The presence of silver nanoparticles added antimicrobial properties to the membrane, which may increase the life time of the membrane. Bacteria contain high concentrations of thiols, that are released into solution during lysis of the bacteria, inducing locally increased concentrations of thiols on the surface of the membrane. Our findings may be used in this technology to optimize the design of such processes balancing performance and duration of the product. For example, PVP coated Ag NPs on these membranes are likely to last longer than citrate coated particles, because they dissolve slower, but that could reduce the antimicrobial properties of the membranes.

In addition, metallic nanoparticles and metal sulfide quantum dots have the potential to be used in medical applications, mostly for detecting and treating infected cells in humans. The surface coating is of paramount importance for the ability of the nanomaterials to “target” the desired cells. Recent studies showed that the uptake of
cysteine coated CdSe(CdZnS) quantum dots from human hepatocellular carcinoma cells was twice the uptake of the same material from human embryonic kidney cells [174].

The production of quantum dots is typically made in media that are impossible to use for medical applications, such as chloroform. Tamang and coworkers showed that InP/ZnS quantum dots produced in chloroform can be transferred to water maintaining their fluorescence properties when coated with cysteine [175]. These findings support the potential use of low molecular weight thiols for the design of improved nanomaterials, but further research is needed to explore this possibility.

Overall, this dissertation work has studied the reactivity of low molecular weight thiols towards metal-based nanoparticles and the effects of this interaction on the particles fate. Our research results demonstrated that improving the scientific knowledge of naturally-occurring nanomaterials may be useful for understanding the fate and transport of manufactured nanomaterials and for designing improved and safer products.
Appendix A Supporting Information for Chapter 2

Figure A1. Intensity-weighted size distribution of the stock ZnS colloid suspension (pH 10.7) measured by dynamic light scattering.

Figure A2. X-ray powder diffraction spectrum of stock ZnS colloids and ZnS reference materials sphalerite (99.9%, <10 μm, Sigma Aldrich) and wurtzite (99.999%, 10 – 150 μm, SPI-CHEM). The data indicated that the stock colloids consisted mainly of sphalerite ZnS due to one primary peak in the range of 26-30°. Peak broadening of the sample spectra (at 29.2°, 48.3°, and 57.1°) indicated that the crystal lattice length dimensions of our synthesized material were 3.4 nm, 4.3 nm, and 5.2 nm (according to the Scherrer formula).
Figure A3. (a) TEM image of the stock ZnS colloids on a copper grid sample holder and (b) element content of particles in TEM image measured by energy dispersive X-ray spectroscopy.
Figure A4. (a) X-ray diffraction spectrum of HgS stock indicating that the particles consisted of cinnabar and/or metacinnabar; (b) TEM image of HgS colloids; (c) EDS spectrum of the particle observed in the TEM image indicating the presence of Hg and S.
Figure A5. (a) Sorption of cysteine on ZnS colloids (50 μM as ZnS) at pH 7.5 (5 mM HEPES buffer), no NaNO₃ added; (b) Recovery of cysteine (35 μM initial concentration) dissolved in pH 7.5 with HEPES buffer and no particles added. The solution was stored in laboratory conditions (e.g., oxic, room temperature).
Figure A6. Aggregation of ZnS colloids (50 μM) suspended in water (pH 7.5) containing (a) 5 mM NaNO₃ and serine (representing the diffusion-limited aggregation regime); (b) 100 μM cysteine and varying NaNO₃ (representing the reaction-limited aggregation regime).
Appendix B Supporting Information for Chapter 4

**Materials.** All chemicals used in this work were ACS reagent grade and purchased from Sigma-Aldrich unless stated otherwise. Barnstead Nanopure-grade water (>17.8 MΩ-cm) was used to prepare all reagents and samples. Trace-metal grade nitric and hydrochloric acids (Fisher Scientific, Pittsburgh, PA) were used to adjust the pH of solutions. Ultrahigh purity nitrogen was utilized for purging oxygen from aqueous samples. Borosilicate glass containers for reagent preparation and storage were acid-washed by soaking overnight in 1 N HCl prior to rinsing three times with Nanopure water.

![Transmission electron microscopy images of Ag NPs](image)

**Figure B1.** Transmission electron microscopy images of (A) citrate-coated and (C) PVP-coated Ag NPs; also shown are particle size distributions determined from TEM images for (B) citrate-coated and (D) PVP-coated Ag NPs.
Figure B2. Time-resolved measurements of nominally dissolved silver with cysteine. Mixtures of 8 μM AgNO₃ and 40 μM (CYS:Ag=5) or 400 μM (CYS:Ag=50) cysteine with 7 mM NaHCO₃ (pH 7.5) and 10 mM NaNO₃ were filtered with 0.025 μm filter membranes.
Figure B3. Concentration of nominally dissolved silver (0.025 μm filter) in mixtures of Ag nanoparticles suspended in solutions containing 7 mM NaHCO₃, 10 mM NaNO₃, and 400 μM cysteine. Total silver concentration was 2.0 μM for Ag-PVP and 8.0 μM for Ag-CIT particles. Both of these concentrations corresponded to the same total surface area in the suspension (13.9×10⁻³ m² L⁻¹). Solution pH was 7.5 to 8.1.
Figure B4. Time-resolved average hydrodynamic diameter of Ag-PVP particles suspended in solution with three different ionic strength values (0.15, 0.05, and 0.03 M). The solutions contained 7 mM NaHCO$_3$, 400 μM cysteine and varying NaNO$_3$ concentrations.
Figure B5. Time-resolved average hydrodynamic diameter of (a) Ag-CIT and (b) Ag-PVP nanoparticles suspended in solutions containing 7 mM NaHCO₃, 10 or 100 mM NaNO₃, and 400 μM cysteine. Particle suspensions are relatively stable at 0.017 M ionic strength and aggregate rapidly (diffusion limited regime – DLR) at 0.107 M ionic strength.
Figure B6. TEM images of Ag-PVP aggregates forming in the diffusion limited regime: (a) 2 hours; (b) 24 hours. Suspensions comprised of 7.6 μM Ag-PVP, 7 mM NaHCO₃, 100 mM NaNO₃ and 0.4 mM cysteine. Solution pH values were 7.5 to 8.1.

Method for TEM imaging
Transmission electron microscopy (TEM) was used to characterize the morphology of Ag-PVP aggregates formed in solution containing 7.6 μM total silver, 7 mM NaHCO₃, 10 mM NaNO₃, and 400 μM cysteine. The solution pH was 7.5 to 8.1. After 2 or 24 h of aggregation, TEM grids were prepared by depositing a 10 μL droplet of sample on a formvar-coated copper TEM grid (Ted Pella). The sample grid was dried for two hours in a HEPA-filtered laminar flow hood. Particles were observed by TEM (FEI Tecnai G² Twin at 160 kV).
Preparation of reference materials for silver XANES analysis

Reference materials for XANES data analysis included PVP- and citrate-coated Ag NPs collected on the membrane filters directly from their stock suspensions. Ag(+I)-cysteine reference materials with 1:1 and 1:2 silver:cysteine molar ratios were prepared by mixing solutions of AgNO₃ (12.8 and 8.8 mM, respectively) and L-cysteine (12.8 and 17.6 mM respectively) and adjusting the pH to 7.5 with 10 N NaOH. We referred to these silver-cysteine references as Ag(CYS) and Ag(CYS)₂ in this study. A silver-citrate reference was prepared from a solution of 9.2 mM AgNO₃ dissolved with 91 mM citrate (at pH 7.5). The dissolved Ag(+I)-ligand reference material solutions were frozen without filtration, freeze-dried, and stored in 2 mL glass vials in a desiccator until XANES analysis. Other silver reference materials for XANES analysis included AgNO₃(s) (Fisher; ≥ 99.7%), AgCl (Fisher Scientific, Hampton, New Hampshire, USA; ≥ 99.0%), Ag₂O (Sigma Aldrich, St. Louis, Missouri, USA; ≥ 99%), and Ag₂S (Alfa Aesar, Ward Hill, Massachusetts, USA; ≥ 99%) that were used directly as powders obtained from commercial suppliers. The reference compounds and freeze-dried samples were deposited on double sided tape as a thin layer, to minimize self-adsorption, and placed in front of the X-ray beam for collection of XANES spectra.
Linear combination fitting of XANES data

Three component linear combination fitting (LCF) of the Ag NP+cysteine sample spectra was performed by for the portion of the data that was 20 eV below and 20 eV above the adsorption edge (3351 eV). The LCF procedure utilized a binary combination of spectra from the reference materials. LCF calculations were not forced to sum to 100%, and as a result, some of the LCF results yielded percentages of model compounds that summed up to 103%. Errors reported for individual components were calculated by the software’s least squares fitting module. Best fits were considered the ones with the lowest R-factor, $\chi^2$, and $\Delta \chi^2$, which are fitting residual parameters defined in the SI section. These parameters are defined from the following equations[176]:

$$R = \frac{\sum \left[ (data - fit)^2 \right]}{\sum \left( data \right)^2}$$

(B.1)

$$\chi^2 = \frac{N_{pts}}{N_{slp}} \sum_i \left( \frac{data_i - fit_i}{\varepsilon_i} \right)^2$$

(B.2)

$$\Delta \chi^2 = \frac{\chi^2}{V}$$

(B.3)
where $i$ is the data points, $\epsilon_i$ the uncertainties at each data point, $N_{idp}$ the number of independent points in the model fit, $N_{pts}$ the number of data points, and $\nu$ the degrees of freedom in the fit.
Figure B7. Silver L3-edge XANES spectra of the model compounds AgNO₃, AgCl, Ag₂O, Ag₂S, Ag(+I) complexes Ag(CYS), Ag(CYS)$_2$, and Ag-citrate. The XANES spectra also include the unexposed Ag nanoparticles (Ag-CIT and Ag-PVP) and the same nanoparticles exposed to CYS for 2 and 24 hours.
Table B1. Linear combination fitting of Ag-CIT samples exposed to cysteine for 2 hours with spectra from the unexposed particles and one of the model compounds or Ag(+I) complexes

<table>
<thead>
<tr>
<th>Ag-CIT + CYS (2 h)</th>
<th>% Ag-NP</th>
<th>% Comp 2</th>
<th>R</th>
<th>$\chi^2_2$</th>
<th>$\Delta\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag$_2$S</td>
<td>95.9 (0.9)</td>
<td>4.9 (0.8)</td>
<td>4.0×10$^{-5}$</td>
<td>13.7×10$^{-4}$</td>
<td>12.1×10$^{-4}$</td>
</tr>
<tr>
<td>Ag(CYS)$_2$</td>
<td>97.2 (0.9)</td>
<td>3.7 (0.8)</td>
<td>4.6×10$^{-5}$</td>
<td>15.5×10$^{-4}$</td>
<td>13.8×10$^{-4}$</td>
</tr>
<tr>
<td>Ag$_2$O</td>
<td>100.0 (0)</td>
<td>1.3 (0)</td>
<td>4.8×10$^{-5}$</td>
<td>16.3×10$^{-4}$</td>
<td>14.4×10$^{-4}$</td>
</tr>
<tr>
<td>Ag(CYS)</td>
<td>99.7 (1.1)</td>
<td>1.7 (1.1)</td>
<td>5.3×10$^{-5}$</td>
<td>18.1×10$^{-4}$</td>
<td>16.0×10$^{-4}$</td>
</tr>
<tr>
<td>AgCl</td>
<td>100.0 (0)</td>
<td>0 (0)</td>
<td>26.2×10$^{-5}$</td>
<td>88.4×10$^{-4}$</td>
<td>78.3×10$^{-4}$</td>
</tr>
<tr>
<td>Ag-citrate</td>
<td>100.0 (0)</td>
<td>0 (0)</td>
<td>26.2×10$^{-5}$</td>
<td>88.4×10$^{-4}$</td>
<td>78.3×10$^{-4}$</td>
</tr>
<tr>
<td>AgNO$_3$</td>
<td>100.0 (0)</td>
<td>0 (0)</td>
<td>26.2×10$^{-5}$</td>
<td>88.4×10$^{-4}$</td>
<td>78.3×10$^{-4}$</td>
</tr>
</tbody>
</table>

Table B2. Linear combination fitting of Ag-CIT samples exposed to cysteine for 24 hours with spectra from the unexposed particles and one of the model compounds or Ag(+I) complexes

<table>
<thead>
<tr>
<th>Ag-CIT + CYS (24 h)</th>
<th>% Ag-NP</th>
<th>% Comp 2</th>
<th>R</th>
<th>$\chi^2_2$</th>
<th>$\Delta\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag$_2$S</td>
<td>92.0 (1.2)</td>
<td>9.4 (1.1)</td>
<td>7.8×10$^{-5}$</td>
<td>26.9×10$^{-4}$</td>
<td>23.8×10$^{-4}$</td>
</tr>
<tr>
<td>Ag(CYS)$_2$</td>
<td>94.6 (1.4)</td>
<td>6.8 (1.2)</td>
<td>9.9×10$^{-5}$</td>
<td>34.2×10$^{-4}$</td>
<td>30.2×10$^{-4}$</td>
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<tr>
<td>Ag$_2$O</td>
<td>100.0 (0)</td>
<td>2.3 (0)</td>
<td>10.1×10$^{-5}$</td>
<td>35.0×10$^{-4}$</td>
<td>31.0×10$^{-4}$</td>
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<tr>
<td>Ag(CYS)</td>
<td>97.2 (1.6)</td>
<td>5.2 (1.6)</td>
<td>11.7×10$^{-5}$</td>
<td>40.3×10$^{-4}$</td>
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<tr>
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<td>0 (0)</td>
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<td>265.5×10$^{-4}$</td>
<td>234.9×10$^{-4}$</td>
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<tr>
<td>Ag-citrate</td>
<td>100.0 (0)</td>
<td>0 (0)</td>
<td>76.9×10$^{-5}$</td>
<td>265.5×10$^{-4}$</td>
<td>234.9×10$^{-4}$</td>
</tr>
<tr>
<td>AgNO$_3$</td>
<td>100.0 (0)</td>
<td>0 (0)</td>
<td>76.9×10$^{-5}$</td>
<td>265.5×10$^{-4}$</td>
<td>234.9×10$^{-4}$</td>
</tr>
</tbody>
</table>
Table B3. Linear combination fitting of Ag-PVP samples exposed to cysteine for 2 hours with spectra from the unexposed particles and one of the model compounds or Ag(+I) complexes

<table>
<thead>
<tr>
<th></th>
<th>% Ag-NP</th>
<th>% Comp 2</th>
<th>R</th>
<th>$\chi^2$</th>
<th>$\Delta\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag(PVP + CYS (2 h))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag$_2$S</td>
<td>90.4 (0.6)</td>
<td>8.9 (0.6)</td>
<td>2.3 x 10$^{-5}$</td>
<td>7.5 x 10$^{-4}$</td>
<td>6.7 x 10$^{-4}$</td>
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<tr>
<td>Ag(CYS)$_2$</td>
<td>91.9 (0.8)</td>
<td>7.2 (0.7)</td>
<td>3.7 x 10$^{-5}$</td>
<td>12.4 x 10$^{-4}$</td>
<td>11.0 x 10$^{-4}$</td>
</tr>
<tr>
<td>Ag(CYS)</td>
<td>91.5 (0.9)</td>
<td>8.6 (0.9)</td>
<td>3.8 x 10$^{-5}$</td>
<td>12.7 x 10$^{-4}$</td>
<td>11.3 x 10$^{-4}$</td>
</tr>
<tr>
<td>AgNO$_3$</td>
<td>99.2 (0.3)</td>
<td>1.1 (0.3)</td>
<td>6.4 x 10$^{-5}$</td>
<td>21.2 x 10$^{-4}$</td>
<td>18.8 x 10$^{-4}$</td>
</tr>
<tr>
<td>Ag$_2$O</td>
<td>99.4 (0.3)</td>
<td>0.8 (0.3)</td>
<td>6.5 x 10$^{-5}$</td>
<td>21.6 x 10$^{-4}$</td>
<td>19.1 x 10$^{-4}$</td>
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<tr>
<td>AgCl</td>
<td>100.0 (0)</td>
<td>0.3 (0)</td>
<td>7.0 x 10$^{-5}$</td>
<td>23.3 x 10$^{-4}$</td>
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</tbody>
</table>

Table B4. Linear combination fitting of Ag-PVP samples exposed to cysteine for 24 hours with spectra from the unexposed particles and one of the model compounds or Ag(+I) complexes

<table>
<thead>
<tr>
<th></th>
<th>% Ag-NP</th>
<th>% Comp 2</th>
<th>R</th>
<th>$\chi^2$</th>
<th>$\Delta\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag(PVP + CYS (24 h))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag(CYS)</td>
<td>91.4 (0.6)</td>
<td>8.8 (0.6)</td>
<td>1.0 x 10$^{-5}$</td>
<td>5.4 x 10$^{-4}$</td>
<td>4.8 x 10$^{-4}$</td>
</tr>
<tr>
<td>Ag(CYS)$_2$</td>
<td>91.3 (0.4)</td>
<td>7.9 (0.3)</td>
<td>1.7 x 10$^{-5}$</td>
<td>2.8 x 10$^{-4}$</td>
<td>2.5 x 10$^{-4}$</td>
</tr>
<tr>
<td>Ag$_2$S</td>
<td>94.4 (0.8)</td>
<td>5.5 (0.7)</td>
<td>1.9 x 10$^{-5}$</td>
<td>10.5 x 10$^{-4}$</td>
<td>9.3 x 10$^{-4}$</td>
</tr>
<tr>
<td>Ag$_2$O</td>
<td>100.0 (0)</td>
<td>0.5 (0)</td>
<td>3.8 x 10$^{-5}$</td>
<td>16.8 x 10$^{-4}$</td>
<td>14.9 x 10$^{-4}$</td>
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<tr>
<td>AgCl</td>
<td>100.0 (0)</td>
<td>0 (0)</td>
<td>4.6 x 10$^{-5}$</td>
<td>28.2 x 10$^{-4}$</td>
<td>24.9 x 10$^{-4}$</td>
</tr>
<tr>
<td>AgNO$_3$</td>
<td>100.0 (0)</td>
<td>0 (0)</td>
<td>4.6 x 10$^{-5}$</td>
<td>28.2 x 10$^{-4}$</td>
<td>24.9 x 10$^{-4}$</td>
</tr>
</tbody>
</table>
Figure B8. (a): Concentration of dissolved cysteine (0.025 μm filter) in mixtures of Ag-CIT and Ag-PVP nanoparticles suspended in solutions containing 400 μM cysteine, 7 mM NaHCO₃ and 10 mM NaNO₃. Solution pH values were 7.5 to 8.1. The total cysteine concentration was measured from the stock solution. (b): Change in dissolved cysteine concentration (Δ[CYS]) (determined from the measured dissolved [CYS] (at the initial time point) and the measured dissolved [CYS] at time t) normalized to the total surface area of the particles at the beginning of the experiment. Errors bars represent 1 standard deviation, estimated by propagating standard deviations of the measured dissolved CYS concentrations.
References


Biography


Mr. Gondikas has received a scholarship from the State Scholarships Foundation (IKY) in Greece. He received the Jeffrey Taub Engineering Excellence Award in 2006.