AN ABSTRACT OF THE THESIS OF

Daniel J. Pike for the degree of Master of Science in Environmental Engineering presented on June 4, 2014

Title: Heteroaggregation of Gold Nanoparticles with Model Colloids and the Influence of Environmental Aqueous Chemistry

Abstract approved:

______________________________________________________

Jeffrey A. Nason

Nanoparticles are becoming increasingly important in numerous technological fields but evidence shows that they can be toxic to many organisms. To predict their fate and transport in the environment, nanoparticle attachment to naturally occurring colloids must be examined. This study investigated citrate-capped gold nanoparticle (Cit-AuNP) heteroaggregation with two model colloids in the presence of natural organic matter (NOM) in different aqueous chemistries. Experimental findings show that the colloidal surface charge relative to Cit-AuNPs strongly influenced attachment in 1mM KCl. Up to 1mg/L NOM stabilized Cit-AuNPs and drastically reduced heteroaggregation, regardless of system pH. In more complex conditions containing divalent cations, NOM also prevented significant heteroaggregation but Cit-AuNP homoaggregation was minimally impacted. This demonstrates that in natural aqueous systems, the presence of NOM stabilizes Cit-AuNPs which enhances transport away from the source of contamination and discourages settling into sediment material.

APPROVED:

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Daniel J. Pike, Author
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1. Introduction

1.1 Motivation

Nanoparticles are receiving greater attention due to their wide range of applications, as well as deleterious effects to environmental and public health. In the last couple of decades, the field of nanotechnology has made a significant contribution to many pertinent scientific fields like medicine, aerospace, construction and food and life sciences [1, 2]. Much of the research in the field of nanotechnology is focused on the benefits and potential uses of nanoparticles. Although it is growing, the existing literature on environmental fate, transport and toxicity is relatively sparse [3]. At some point in their lifecycles in industrial and consumer products, engineered nanoparticles will be transported into the natural environment [3-6]. This could be accomplished through passage into waste water treatment plants (WWTPs), accidental spills and leaks, aging of products that contain nanoparticles, etc. Once released, nanoparticles are subject to a myriad of different physical and chemical modifications [7]. Additionally, they will interact with natural organic matter (NOM) or become incorporated into the tissues of living organisms [2, 5, 7-10]. Having an understanding of nanoparticle behavior in environmental systems and WWTPs is critical to predicting exposure and toxicity.
In environmental systems, nanoparticles can undergo a physical transformation known as aggregation. Collisions and attachment of like particles is known as homoaggregation. These events can also take place between unlike particles and that process is termed heteroaggregation. Aggregation of nanoparticles can have a profound impact on their transport and reactivity in the environment. Particles that stay in solution tend to be more bioavailable and are subject to transport over a greater distance. Nanoparticles that aggregate are more likely to settle out of the water column. They may become incorporated into sediment material or into the cells of living organisms. Bacterial communities that dwell on the floors of water bodies may also be susceptible to toxicity effects from certain nanoparticles [11]. Examining the mechanisms and conditions in which nanoparticles aggregate is an important prerequisite to predicting their transfer and toxicity in the environment.

1.2 Problem Statement

Inevitably, nanoparticles will be released into environmental systems and may require monitoring or remediation measures. To accurately predict their transport and reactivity in natural systems, efforts should be made to analyze aggregation behavior. Although nanoparticles are expected to interact with natural colloids, the aqueous conditions favoring heteroaggregation are unclear. Investigating nanoparticle attachment with these materials is required to accurately assess environmental fate and
transport. Additionally, NOM has been shown influence particle stability. Further research is needed to better understand when NOM enhances stability and thus nanoparticle heteroaggregation and environmental behavior.

1.3 Objectives

This paper aims to build on previous work that examined citrate-capped gold nanoparticle (Cit-AuNP) heteroaggregation with model hematite and silica colloids in a simple aqueous matrix. To more accurately assess nanoparticle fate and transport in the environment, heteroaggregation was analyzed in the presence of additional constituents. Specific objectives of the research are as follows:

1. To determine the role of environmentally relevant ionic strength and hardness on nanoparticle aggregation in the presence of model colloids.

2. To investigate how the presence of natural organic matter (NOM) influences Cit-AuNP heteroaggregation behavior.

3. To demonstrate the importance of colloid surface charge character on Cit-AuNP heteroaggregation.
1.4 Approach

These objectives were accomplished using Time-Resolved Dynamic Light Scattering (TR-DLS) which is a well-established analytical technique. This technique uses light scattered by particles in solution to calculate the average particle diameter, up to several microns. Under stable conditions, the average particle size remains constant. When particles are destabilized they form aggregates which increase the average particle size. TR-DLS also allows the user to calculate other useful parameters such as the initial aggregation rate and the extent of aggregation over a desired time period.

In an environmental remediation scenario, it would be beneficial to know the fraction of released nanoparticles that associate with colloidal material versus that which remains freely suspended. To do this, a previously devised methodology has been applied, which utilizes a nylon syringe filter to separate gold nanoparticles from those that adhere to model colloids. The samples are then analyzed using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Comparing the relative amounts of gold in the filtrate and retentate helps us to realize this goal.

The following chapter is a review of colloidal interaction theory and application to nanoparticles. The importance of organic matter in heteroaggregation mechanics and previous studies looking at the toxic effects of nanoparticles on ecological systems are also explored. Chapter 3 describes the materials, analytical
instruments and techniques that were utilized. Chapter 4 presents experimental results. Chapter 5 details conclusions that can be drawn from this work and offers suggestions regarding future research on this topic. Additional data is presented in the appendix for reference.
2. Background

2.1 Nanoparticles and Environmental Impact

Nanotechnology can be defined as the manipulation or application of matter with at least one length dimension less than 100 nm, called nanomaterials. [2, 5, 7, 12-14]. These materials have relatively high surface area to volume ratios, which allows them to achieve the same reactivity as their bulk counterparts, but with less required mass. This may result in lower cost associated with using a particular material. Certain nanomaterials also have unique electrical properties at the nano-scale, such as carbon nanotubes. These, along with other carbon-based materials, are being utilized in a wide variety of fields like semi-conductors, medicine and energy storage [11]. Additionally, some nanoparticles can be manipulated into many different geometric forms which can impart or enhance a desired functionality. An illustration of this is the evidence that both the size and shape of silver nanoparticles is important to bactericidal function. Martinez et al. found that 7-nm particles were more effective than their larger counterparts against E. coli and S. aureus while Pal et al. concluded that triangular silver nanoparticles performed better than comparably sized rods or spheres [15, 16].

The expanding use of nanoparticles can be traced back to physicist Richard Feynman’s assertion that the ability to manipulate matter on the atomic scale would lead to great technological advances [12]. As microscopy and other instruments have
developed, wide scale use of nanotechnology became possible. One study published in 2012 reports that titanium dioxide, one of the most widely used nanoparticles, is produced on the order of tens of thousands of tons per year [17, 18]. Applications include cosmetics, sunscreens, paint pigments, food additives and catalytic technology, and others [19, 20]. Silver nanoparticles are also being widely applied in consumer products and medical devices for their antimicrobial properties [21]. Nano-scale metal oxides such as iron oxide are becoming popular in some environmental remediation cases [3]. Uses for nanoparticles have been found in many industries and it is estimated that over $2 trillion worth of goods utilize nanotechnology [12]. As cheaper and more efficient means of production are developed, nanotechnology will likely continue to play a pivotal role in many facets of society.

Engineered nanoparticles are often synthesized with a core material and ligands attached to their surface known as capping agents. Depending on the core properties, capping agents can vary widely in size and functionality. It has been demonstrated that although size and shape may be important to biological uptake, nanoparticle reactivity is primarily due to surface chemical species. This capping agent is also an important factor in environmental aggregation behavior. The capping agent of a nanoparticle often dictates its ecological toxicity. One study that utilized zebrafish embryos investigated gold nanoparticles of similar size, having three different surface functionalities. The authors reported that two of these engineered particles induced cellular damaged whereas 2-(2-(2-mercaptoethoxy)ethoxy)ethanol
(MEEE) coated gold particles showed no detectable response [22]. Another paper by Goodman et al. examined the ability of gold nanoparticles with positively and negatively charged capping agents to lyse red blood cells as well as *E. coli* bacteria. The results indicated that the cationic particles interacted with cell membranes and ruptured cells more extensively than the anionic variety [23].

There is also a growing collection of data suggesting that some of these materials may adversely affect human health and the environment. One well-studied example is carbon nanotubes. Carbon nanotubes have a long, thin configuration like asbestos fibers. It has been shown that inhaled nanotubes can accumulate in lung tissue, eventually leading to chronic lung ailments [9]. Demonstrations have shown that chronic inhalation exposure to other nanoparticles like titanium dioxide and iron oxide can lead to similar outcomes in rats [16, 24].

At some point in their life cycle, products containing nanoparticles are consumed and ultimately disposed of. During this time, it is inevitable that some of these particles will be transported into the environment by way of atmospheric discharge, spills and leaks, through WWTPs or some other industrial process. Releases during production or transport are also possible. Much of the research concerning nanoparticle transport in wastewater treatment plants look specifically at silver nanoparticles because of their bactericidal effects [13, 21]. Some research has suggested that silver nanoparticles could threaten beneficial microbes and impair
normal operations within the plant [3]. Associated silver nanoparticles may impair microbial ecology in soil if sludge from the facility is applied for agricultural use [3].

WWTPs are not usually built to remove contaminants in the size range relevant to nanoparticles. They often utilize sedimentation to remove small particles that can settle out of solution in a reasonable time. Although they are effective at degrading and removing contaminants like pathogens, organic substrates and large debris, particles less than 100 nanometers in length will not likely fall out of solution [3]. However, there is evidence that certain nanoparticles can either sorb to larger materials or become incorporated into biomass [25]. As a result, nanoparticles that remain stabilized throughout the treatment process and avoid microbial uptake will be released into receiving water bodies.

It has also been observed that many other nanoparticles besides silver can be detrimental to natural ecosystems [26-28]. The mechanisms and specific targets of toxicity are also diverse. For example, exposure to certain classes of nanoparticles can encourage the generation of reactive oxygen species which can damage DNA and inhibit cellular metabolism [29].

A growing body of literature is also highlighting the detrimental effects of nanoparticles on both plant and animal life [1]. During environmental transport, nanoparticles may move through more than one environmental compartment such as the atmosphere, surface water, ground water, and biomass. Because of this, toxicity research cannot be isolated to a single compartment. A review by Klaine et al. gives
several examples of work conducted on saltwater and freshwater organisms as well as soil microbes and plant species [11]. Interestingly, the authors also mention the uptake of nanoparticles in agricultural crops such as corn and lettuce. Some effects on germination have been reported, but additional concern may arise if sustenance crops are accumulating nanoparticles which would then lead to human exposure [11].

Studies are starting to examine the consequences of nanoparticle exposure to a variety of organisms across many ecosystems. Because engineered nanoparticles can present hazards to ecosystems, it is important to study how nanoparticles behave in the environment so that we can better predict where they will go and in what form. This will allow for accurate prediction of the risk that these materials pose to the environment.

2.2 Gold as a model

The work detailed here uses citrate-capped gold nanoparticles (Cit-AuNPs) as a model system. This model particle is advantageous for several reasons. As previously mentioned, reactivity for many nanoparticles is determined by its capping agent. AuNPs can be coated with a range of chemical moieties, which is ideal for investigating how capping agents affect aggregation activity. Gold also has a vast number of applications and is already produced in large quantities. In the medical field, AuNPs are a promising material for use in anti-cancer therapies and imaging techniques, genetic diagnostics, and others. AuNPs also have interesting physical
properties that allow them to be used in relatively benign photothermal treatment against tumor cells \[30\]. They are relatively inert in biological systems, and exhibit minimal toxicity to human tissue \[31\]. Not only does this make them suitable for biomedical purposes, but working with gold particles presents a low health hazard in the lab setting. Moreover, AuNPs with similar sizes to those used in this work have a plasmon resonance band at approximately 517nm. This allows for analysis using UV-visible spectroscopy \[32\]. These qualities make AuNPs a relevant and useful platform for the study of nanoparticle fate and transport in aquatic systems.

2.3 Particle Behavior and the Electrostatic Double Layer

Derjaguin-Landau-Verwey-Overbeak (DLVO) theory has been used to describe the interactions of colloidal particles. In this case, colloids are understood to have at least one dimension measuring less than one micron (1,000 nm) \[7, 11\]. The original work from these researchers proposes that the probability of particles attaching to one another is determined by two forces. One of these is the Electrostatic Double Layer (EDL) forces which are derived from the surface charge of a particle. Species of opposite charge experience attractive electrostatic forces while those with like charges are repulsive. The second type is van der Waals (vdW) interactions. Attractive van der Waals interactions are only felt at very close range. When particles move to within a few nanometers of one another, areas of both slightly negative and
positive charge generated from movement of electrons create an attractive force.
Calculating the vdW attractive energy for two particles is a function of both their size and an intensive quality known as the Hamaker constant [33, 34]. The sum of these forces gives the energy barrier to attachment. A high energy barrier indicates that particles are unlikely to collide and attach, whereas a low value suggests that attachment is energetically favorable.

Charged particles can be modelled with two layers extending from the surface into the bulk solution. The first layer is known as the compact layer, which encompasses ions very close to the solid particle. Ions with charges opposite to those at the surface are more concentrated in the diffuse layer while those with a like charge are less concentrated. The diffuse layer extends past the compact layer and is longer than the compact layer. Zeta potential measurements describe the electrical potential at the boundary where the compact and diffuse layers meet [35, 36].

The charge on a particle’s surface is a result of both the presence of chemical functional groups and the pH of its environment. In natural aqueous systems, particles can have a wide variety of organic and inorganic species present on their surfaces. Examples include components like proteins, polymers, acids and bases, or inorganic moieties like sulfates, phosphates, and carbonates. Metallic surfaces are also possible. The system pH has a significant effect on charge because it determines the availability of free H\(^+\) and OH\(^-\) ions in solution. At low pH, acidic groups remain primarily protonated. On the other hand, these species are able to release protons into the bulk
solution in alkaline conditions, which imparts a negative charge. This surface charge is principally responsible for particles’ resistance to aggregation and is associated with its diffuse layer. Materials with like charges are repulsed by each other, while those with opposite charges experience an attractive force. Since particles with high energy barriers are unlikely to come into close contact, aggregation is prevented.

When dealing with nanoparticles, other mechanisms besides those delineated in DLVO theory should be considered. There are also assumptions in DLVO theory that are not applicable in some systems containing nanoparticles. To use DLVO principles, particles are assumed to be spherical [7]. It has been pointed out that nanoparticles can take many shapes which violate this assumption. Additionally, they are often modified with surface functional groups that may participate in acid/base or redox reactions, for example. These surface coatings can also degrade or be replaced by other chemicals in the environment [6, 7]. Nanoparticles may also be capped by large macromolecules, like polypeptides, which provide stabilization through steric hindrance. Interactions with organic substances in the environment can also enhance or inhibit aggregation. This is delineated in greater detail in later sections. Other interactions such as redox and photodegradation may also be important processes in some instances [2].
2.4 Particle Destabilization

The ionic strength ($I$) is an important characteristic of an aqueous system. It is a measure of free ions in solution and is calculated as:

$$I = \frac{1}{2} \sum_{\text{all ions}} c_i * z_i^2$$

Where $i$ represents a particular ion, $c$ is concentration (M), and $z_i$ is the charge of ion $i$. According to the principles laid out in DLVO theory, ionic strength will influence the effective range for a particle’s diffuse layer. A diffuse layer with a greater characteristic length will prohibit particles from coming into close contact where attractive van der Waals forces can be felt. In the simplified Debye-Hückel form, the variable, $\kappa$, of the EDL is measured in units of nm$^{-1}$. Its inverse ($\kappa^{-1}$) is the characteristic length of the diffuse layer. The simplified form is given below:

$$\kappa = 3.288\sqrt{I}$$

Although not always applicable, the Debye-Hückel approximation shows that ionic strength is related to $\kappa^{-1}$. As the ionic strength of a solution increases, the length of the diffuse layer decreases, which diminishes repulsive effects [7]. Reducing the characteristic length by means of increasing the ionic strength of a system is known as diffuse layer compression.

Some treatment processes utilize other methods to induce particle destabilization. Adsorption and charge neutralization is commonly applied in drinking water systems. Salts such as aluminum sulfate $\text{Al}_2(\text{SO}_4)_3$ and ferric sulfate $\text{Fe}_2(\text{SO}_4)_3$
are added to a system, where the metal salt dissociates to form hydroxide complexes. These chemicals then adsorb to the particle surfaces and decrease their charge. Other reagents such as organic polymers are also very effective in this task [35, 36].

Polymers destabilize particles by means of interparticle bridging as well. For effective bridging, polymers with relatively long molecular chains are employed and may also exhibit positive or negative charge [35]. The reagent adsorbs to several particles which intermingle with other polymer-particle complexes to yield large, settleable masses. Introducing divalent cations such as calcium or iron can also enhance this process. They facilitate attachment when the particles in solution and added polymer are both negatively charged.

Introducing metals, like aluminum or iron, can also aid in particle removal by a mechanism known as sweep flocculation. When a large amount of multivalent metals are added to solution, they attach to particles to form precipitates. With sufficient time, precipitates adhere to one another and create large flocs that can be separated from the bulk solution.

2.5 Heteroaggregation

The fate and transport of nanoparticles in the environment is greatly influenced by their tendency to aggregate. When destabilized, nanoparticles are able to collide and attach to other materials in solution. Homoaggregation describes the process where particles interact with like particles. An example of this occurs when Cit-
AuNPs aggregate with each other. Most of the nanoparticle fate and transport literature so far has examined this type of aggregation [37]. Particles undergo heteroaggregation when they collide with and attach to a different class of particle. In environmentally relevant conditions, heteroaggregation involving nanoparticles and colloids could take place. In fact, this type of interaction is more likely to occur under most circumstances because discharged nanoparticles will probably be found at a significantly lower concentration than colloids [7, 11]. For example, one model used to predict exposure found that common nanoparticle materials like gold, silver and carbon fullerenes would be present in water at less than 1 µg/L, while freshwater concentrations of colloids can exceed 10 mg/L [6, 11]. Because natural colloids are more widely distributed and present in greater concentrations throughout natural aqueous systems, heteroaggregation is expected to be more relevant in nanoparticle fate and transport than homoaggregation.

Nanoparticle aggregation in the environment can be influenced by many factors. One of these is naturally occurring organic matter. It is now well-established that the quantity and composition of organic matter in the environment can have a dominating impact on interactions between particles [14, 27, 38, 39]. The physical and chemical properties of nanoparticles and larger colloidal materials are also important. Parameters such as size, shape, core material and surface functionalities should be considered when analyzing aggregation [34]. Some research has also looked at heteroaggregation between two engineered nanoparticles. Afrooz et al. used DLS to
study homoaggregation and heteroaggregation of acrylic acid-stabilized gold nanoparticles and single-walled carbon nanotubes. The authors found that heteroaggregation increased with increasing ionic strength. The presence of SRHA also aided attachment when divalent Ca\(^{2+}\) was also in solution [40].

Understanding the mechanisms and conditions by which nanoparticles heteroaggregate is critical to determining how they are transported in the environment. This, in turn, plays an important role in identifying and quantifying the risk particles may pose. There are several effects that should be considered as a result of aggregation. One of the advantages to nano-scale materials is the greater available reactive surface area to volume ratio than in bulk. However, this quality is partially lost when nanoparticles destabilize and form larger aggregates. This could be detrimental in an industrial setting, but may be desirable if potentially toxic nanoparticles are released. The reduction in available surface area may present a lower risk to susceptible organisms [2].

Additionally, nanoparticles that heteroaggregate will be more likely to settle out of solution. According to Stokes’ Law, nanoparticles experience a very small downward force due to gravity and will not settle out of solution in a relevant time frame [3]. Because settling velocity is heavily dependent on particle size, nanoparticles that are destabilized and interact with larger colloids would be expected to undergo more rapid sedimentation. This decreases the concentration of nanoparticles in the aqueous phase, but increases their prevalence in sediment
material. When conditions of a water body receiving nanoparticles discourage aggregation, they may be transported over great distances. Understanding aggregation behavior is critical to make an accurate risk assessment for nanoparticle exposure in the environment.

2.6 Aggregation Measurement Methods

Many analytical techniques now exist to characterize the aggregation state of nanoparticles. Several types of imaging, such as Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM), are commonly used to visually inspect particle shape, size and extent of aggregation [6]. However, caution should be taken when using electron microscopy techniques because nanoparticle aggregation behavior can be altered during sample preparation [41]. This type of imaging analysis can also be sensitive to particle interactions with impurities. Many studies available in the literature have relied on DLS as a primary analytical technique [6, 40, 42, 43]. DLS is advantageous over other techniques in several ways. It is a non-destructive instrument, so prepared samples can be analyzed multiple times. Sample sizes are also relatively small, which consumes test materials at a slower rate. This method also requires minimal time for sample analysis. DLS is also an appropriate analytical choice because it can accurately detect and measure particles from approximately 5 nm up to several microns [6]. However, DLS systems have weaknesses that prevent them from more extensive use. Samples should be monodisperse, as a wide size
distribution can skew measurements of average particle diameter. Constituents are also assumed to be spherical, which is not true for many engineered nanoparticles. Taking these qualities into consideration, it was determined that DLS is a reasonable analytical method for the materials in this research [41].

As previously mentioned, heteroaggregation is more environmentally relevant than homoaggregation, but both types of experiments were carried-out using TR-DLS in this work. DLS uses a laser to emit light through an aqueous sample, to a detector at a fixed angle and distance. Particles in the system scatter this incoming light before reaching this detector. The additional time required for light to reach the detector is translated by the instrument to a diffusion coefficient and subsequently to hydrodynamic diameter according to the relationship:

\[ D = \frac{(k_B \times T)}{(3\pi\eta(t)d)} \]

Where \( D \) is the diffusion coefficient, \( k_B \) is the Boltzmann Constant (1.381*10^{-16} ergs/deg), \( T \) is temperature in Kelvin, \( \eta(t) \) is the viscosity of the solvent and \( d \) represents particle diameter.

### 2.7 Natural Organic Matter

When studying the physical behavior of nanoparticles in environmentally relevant conditions, organic matter needs to be considered. NOM is a broad term that describes a class of organic macromolecules that primarily consists of decaying plant and animal matter. These often contain carboxylic and phenolic acids and typically
lower the pH of a system as a result [44]. In this study, Suwannee River natural organic matter (SR-NOM) was used to model the organic composition found in natural systems. It is composed of both humic and fulvic acids [42]. These are types of humic substances found in natural systems.

Although physically similar, humic and fulvic acids are differentiated by several factors. Fulvic acids tend to have greater oxygen content and lower concentrations of aromatic rings [45]. In some instances, organic matter may be a desirable freshwater constituent. Previous work has argued that organic matter is capable of reducing the toxicity of an environmental pollutant [32]. However, in many circumstances, NOM is also a hindrance. NOM can clog filtration membranes and aid in the creation of disinfection byproducts following common water treatment operations. NOM has been investigated extensively and many studies have shown that it can have a profound impact on nanoparticle stability, fate and transport, and toxicity [32, 39, 46, 47]

Several studies have demonstrated that NOM coats natural colloids and enhances particle stability [48-50]. Zhang et al. investigated the stability of metal oxide nanoparticles in the presence of organic matter. They calculated the increase in the net energy barrier to particle attachment as a function of NOM concentration [5]. Zeta potential measurements indicated that NOM coated the surface and imparted a negative surface charge for most of the tested nanoparticles. Another study by Stankus et al. included experiments on gold nanoparticles with a variety of capping agents. The
addition of 5mg/L Suwannee River Humic Acid (SRHA) as total organic carbon (TOC) consistently resulted in increasingly negative zeta potential, regardless of capping agent charge [8].

In some instances, NOM destabilizes particles and enhances aggregation kinetics. NOM can participate in interparticle-bridging when divalent cations are also in solution [5, 8, 43]. Since NOM contains an array of acidic functional groups, it exerts a negative charge under relevant pH conditions. It’s believed that multivalent cations form electrostatic bonds with NOM-coated nanoparticles. This results in destabilization and formation of aggregates. Although NOM tends to stabilize particles, the inclusion of cations like calcium and magnesium may have a destabilizing effect.

2.8 Previous Heteroaggregation Investigation with Hematite

The experiments in this thesis build off of the work presented by Smith [51]. That study examined the heteroaggregation behavior of Cit-AuNPs and hematite colloids. The author used 10 mg/L hematite in all samples. Suspensions containing up to 3 mg/L Cit-AuNPs were tested, and the ionic strength was held constant at 1mM KCl. Smith demonstrated that 0.6 mg/L Cit-AuNPs caused the greatest extent of aggregation with hematite colloids in 1mM KCl solution at pH 6. It was shown that this amount of Cit-AuNPs resulted in approximately 40% of maximum surface
coverage and was hypothesized to create regions of positive and negative surface charge, according to the electrostatic patch model [51, 52].

At lower Cit-AuNP concentrations, hematite particles retained positive surface charge character which resulted in less extensive heteroaggregation. When the concentration of Cit-AuNPs increased above 0.6 mg/L, the hematite particles became increasingly coated with negatively charged Cit-AuNPs, ultimately reversing the surface charge and stabilizing the heteroaggregates via electrostatic repulsion. Relatively low concentrations of Cit-AuNPs resulted in complete attachment to hematite. As the concentration increased, more Cit-AuNPs heteroaggregated until the hematite was completely coated. Beyond that point, excess Cit-AuNPs remained unattached in solution. At pH 8, only 0.35 mg/L Cit-AuNPs are required to achieve the highest aggregation extent. The hematite has a less positively charged surface which attracts a lower number of Cit-AuNPs.

These findings demonstrated how Cit-AuNPs heteroaggregate with hematite colloids in a relatively simple system containing a monovalent salt and discussed relevant mechanisms. Environmental implications were also discussed; Cit-AuNPs attached extensively to the oppositely charged model colloid. Additionally, complete attachment is predicted at environmentally relevant concentrations. The author makes several suggestions for future work, such as investigating the effects of NOM, expanding colloid variety, nanoparticle capping agents and salt content.
This work also examines the heteroaggregation of Cit-AuNPs with silica colloids that carry the same surface charge as Cit-AuNPs in relevant aqueous conditions. The role of organic matter in Cit-AuNP heteroaggregation with both model hematite and silica is assessed, and more realistic environmental aqueous conditions are also investigated by including a wider range of monovalent and divalent electrolytes.
3. Materials and Methods

3.1 Electrolytes

Potassium chloride (KCl) was used to adjust the ionic strength of test samples. Very hard standard synthetic freshwater was prepared using 480 mg of calcium sulfate (CaSO₄·2H₂O) and magnesium sulfate (MgSO₄), approximately 770 mg sodium bicarbonate (NaHCO₃) and 32 mg of KCl. All electrolytes were of ACS reagent-grade. Upon dissolution, stock solutions were filtered with 0.2 μm nylon syringe filters to remove remaining suspended particles. For all experiments, distilled deionized (DDI) water from an ELGA® Purelab® Ultra (VWS, UK) was used, with a measured electrical resistance of 18.2MΩ-cm.

3.2 Model Colloids

Colloidal silica particles were purchased from nanoComposix (San Diego, CA) in a 10.6 mg/ml stock solution and suspended in DDI water. According to the manufacturer, the product is monodisperse, with an average hydrodynamic diameter of 150.4± 16.5 nm as measured by a Malvern Zetasizer Nano ZS. This measurement was supported by DLS analysis using a 90Plus Particle Size Instrument (Brookhaven Instruments Corporation, Holtsville, NY). An aliquot from the stock was diluted in DDI water to a concentration of 10 mg/L silica and acidified with HCl to pH 6 before
To maintain consistency with work from Smith, this colloid concentration of 10mg/L was used for all applicable experiments in this study. The zeta potential of 10mg/L silica in 1mM KCl was also calculated as $-32.65 \pm 1.80$ mV at pH 6 and $-46.40 \pm 8.18$ at pH 8. These results are fairly close to those reported by Metin et al. [53].

Previously synthesized hematite colloids were also utilized. The synthesis method used to produce this material is described by Smith [51]. The stock solution has a gravimetrically determined concentration of 1.6 g/L and is kept at room temperature. Zeta potential analysis was conducted in 1 mM KCl solution at a concentration of 10mg/L hematite. pH adjustments were made using HCl or KOH. Results showed that hematite particles have a positive surface charge under most environmentally relevant pH conditions, with an isoelectric point (IEP) at approximately pH 9.7. The average particle size was measured using the same DLS instrument as described above. Prior to use, the hematite stock was sonicated for about one minute using an Ultrasonics Cleaner (VWR International, Radnor, PA) to break-up aggregates and homogenize the solution.

Before investigating heteroaggregation behavior, it is instructive to understand the homoaggregation kinetics for the particles of interest. An established method to describe this behavior is to calculate the attachment efficiency, $\alpha$, which indicates the probability that two particles approaching one another will successfully attach [27,
According to Chen et al. the attachment efficiency can be calculated by the following equation:

\[ \alpha = \frac{(k_{11})}{(k_{11})_{fast}} \]

Where \( k_{11} \) is the aggregation rate constant for primary particles and \((k_{11})_{fast}\) is the diffusion-limited rate constant. \( k_{11} \) can be calculated from the equation describing the initial aggregation rate for a population of particles:

\[ \left(\frac{dN_1}{dt}\right)_{t\to0} = -k_{11} * N_0^2 \]

\((dN_1/dt)\) is the rate of primary particle consumption, and \(N_0\) is the initial particle concentration. The calculation of \(k_{11}\) assumes that the initial particle concentration is kept constant for each sample [38].

From the previous discussion, it may be recalled that the EDL of a particle is compressed as the ionic strength of the solution is increased. EDL compression makes successful particle collisions more likely and results in a higher initial aggregation rate. When the particle surface charge is sufficiently compressed, the energy barrier to interaction becomes zero, allowing particles to homoaggregate at a diffusion-limited rate. The ionic strength at which this energy barrier is eliminated is known as the critical coagulation concentration (CCC).
3.3 Gold nanoparticles

Citrate-capped gold nanoparticles were purchased from nanoComposix. The diameter as measured by TEM and reported from the manufacturer is 11.8±0.8 nm. The mass concentration was also given as 0.0516 mg/ml as gold, with a particle concentration of 3.0*10^{12} particles/ml. Excess citrate in solution was removed through repeated centrifugation and re-suspension using 10 KDa Amicon Ultra centrifugal filters as detailed by Smith [51]. Additionally, Smith demonstrated the Cit-AuNPs were stable in 1mM KCl at pH 6.1 and 8 [51].

3.4 SR-NOM

Suwannee River Natural Organic Matter was purchased from the International Humic Substances Society (St. Paul, MN). The isolate was suspended in DDI water at an initial concentration of approximately 40 mg/L as dissolved organic carbon (DOC). This solution was acidified to pH 4 and stirred for 24 hours, away from light. The stock was filtered with a 0.2 µm nylon syringe filter (VWR International) and stored at 4°C. The DOC concentration was determined using a Shimadzu TOC-VCSH following EPA Method 415.1 for carbon analysis in several water categories. After analysis, the solution was refrigerated at 4°C.
3.5 Standard Test Water

To study the aggregation behavior of gold nanoparticles in samples that more closely mimic environmental conditions, standard synthetic freshwater was created, as outlined in the EPA’s “Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition” (2002). Briefly, two liters of “very hard” water were prepared using dry salts and DDI water in volumetric glassware. The solution was then stirred for several hours until completely dissolved and subsequently aerated overnight.

The EPA standard water was tightly capped in a Nalgene™ bottle and refrigerated at 4°C. In experimental samples, the very hard stock water was diluted with DDI water to achieve softer conditions according to recipes in the EPA Methods text. The ionic strength, pH and water hardness of the five conditions used in this study are presented in Table 3-1.

Table 3-1: Ionic strength, pH and water hardness for the five dilutions of EPA standard test water utilized

<table>
<thead>
<tr>
<th>Hardness Class</th>
<th>Ionic Strength</th>
<th>pH</th>
<th>Hardness (mg/L as CaCO₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)</td>
<td>(mM)</td>
<td>(-)</td>
<td>(mg/L as CaCO₃)</td>
</tr>
<tr>
<td>Very Soft</td>
<td>0.57</td>
<td>7.35</td>
<td>10.47</td>
</tr>
<tr>
<td>Soft</td>
<td>2.26</td>
<td>8.09</td>
<td>41.90</td>
</tr>
<tr>
<td>Moderately Hard</td>
<td>4.52</td>
<td>8.41</td>
<td>83.80</td>
</tr>
<tr>
<td>Hard</td>
<td>9.05</td>
<td>8.58</td>
<td>167.60</td>
</tr>
<tr>
<td>Very Hard</td>
<td>18.09</td>
<td>8.60</td>
<td>335.19</td>
</tr>
</tbody>
</table>
3.6 Cit-AuNP Fractionation and ICP Analysis

The fraction of AuNPs associating with colloids was determined using a filtration method developed by Smith [51]. 80 nanometer pore size polycarbonate filters (Nuclepore Track-Etched Membrane, GE Healthcare) were used with a Luer Lock-capable 20 ml syringe. Samples were prepared in 14 ml centrifuge tubes and allowed to sit for approximately 30 minutes. Half of this volume was then extracted and placed in a polystyrene culture vial to serve as a control for ICP analysis. The rest of the solution was filtered using the apparatus described above. Filtrate was collected and then the filter was removed and placed in a separate container. This filter was re-suspended with 1 ml DDI water, acidified with aqua regia (HCl: HNO₃ ratio of 3:1) and sonicated for one minute. After 30 minutes, additional water was added and the sample was sonicated again to extract remaining metal from the filter. The solution was then decanted and stored in a polystyrene culture vial. All samples were acidified with an aqua regia mixture of 5% by volume. The acid digestion procedure was slightly modified in a later experiment. The details are presented in the appendix. Metal content was quantitatively analyzed using ICP-OES (Teledyne, Leeman Prodigy, Hudson, NH).
3.7 Time-Resolved Dynamic Light Scattering

In homoaggregation experiments, samples were prepared by first adding DDI water to a 4 ml polystyrene cuvette (Brookhaven Instruments) and pipetting the appropriate amount of colloid or nanoparticle. Next, the pH was modified using KOH or HCl. SR-NOM addition would follow, if desired. Finally, ionic strength was adjusted as necessary using stock KCl or CaCl$_2$. For studies in standard test water, the model colloid or nanoparticle was pipetted into DDI water. Stock SR-NOM was pipetted before ionic strength adjustment with very hard water in applicable samples. The appropriate volume of very hard water was added instead of KCl to achieve the desired final hardness, and the starting time was recorded. All homoaggregation experiments used a colloidal concentration of 10 mg/L and a Cit-AuNP concentration of 1 mg/L. Runs included 120 measurements over a 32-minute time period.

Constituents were introduced differently for heteroaggregation experiments. Approximately 90 µl of model colloid stock solution was added to DDI water first and initial sizing measurements were made in triplicate (one-minute data accumulation). Acid or base addition was conducted next, followed by addition of SR-NOM, if required. In tests where KCl was the only added salt, ionic strength alterations were made next and followed by another triplicate sizing measurement. Finally, the Cit-AuNPs were introduced to a beginning the heteroaggregation trial. TR-DLS runs were conducted over the same time period as the homoaggregation tests. Very hard water
was the last constituent introduced in standard test water trials in order to collect initial sizing measurements prior to aggregation.

Aggregation data can be summarized from raw TR-DLS output by calculating the extent of aggregation. The extent of aggregation \( (D_{h,30}/D_{h,0}) \) is the calculated average hydrodynamic diameter at the end of the testing period, normalized by the initial hydrodynamic diameter of the hematite colloids. Other representations of particle aggregation have been utilized in the literature, such as the initial aggregation rate \([8, 40]\) and attachment efficiency \([43]\).

3.8 Zeta Potential Titrations

Characterization of colloidal surface charge was accomplished by measuring the sample’s electrophoretic mobility using ZetaPALS software and the 90Plus Particle Size Analyzer described above. Samples were prepared in the same polystyrene cuvettes utilized for TR-DLS. The order of constituent addition proceeded as follows: DDI water, model colloid, KCl, SR-NOM if applicable. Experiments were conducted with 10mg/L of either hematite or silica, and 1mM KCl.

Measured electrophoretic mobility was converted to zeta potential by using the Hückel approximation for small values of \( \kappa a \) with a Henry correction according to the equation:
\[
\mu_e = \frac{2e\zeta}{3\eta} * f(\kappa a)
\]

Where \( \mu_e \) is the mobility, \( \zeta \) is zeta potential (mV), \( \eta \) is the solvent viscosity, and \( e \) is permittivity. \( \kappa a \) is the Henry correction- a dimensionless product of the inverse characteristic length of the diffuse layer, and particle radius [54].

### 3.9 UV-Visible Spectroscopy

Before Cit-AuNPs could be utilized for heteroaggregation experiments with model colloids, excess citrate had to be removed from solution. The manufacturer shipped the product in a 2mM sodium citrate buffer solution. Smith found that this interfered with heteroaggregation experiments by de-stabilizing the hematite colloids [51].

A calibration curve was generated for UV-visible spectroscopy analysis to determine the gold concentration in the cleaned Cit-AuNP solution (see appendix). Following centrifugation, the recovered Cit-AuNPs were diluted to an estimated concentration of 1.5mg/L and processed using a Genesys 10S UV-Visible spectrophotometer (Thermo Scientific, Waltham, MA). 12nm gold nanoparticles have a peak plasmon resonance at a wavelength of 517 nm. The absorbance of the cleaned stock solution at this wavelength was measured and used to calculate the Cit-AuNP concentration.
4. Results

Chapter 4 presents and discusses experimental work from this study. It is broadly separated into two sections. Section 4.1 looks at Cit-AuNP heteroaggregation with hematite. Section 4.2 examines laboratory studies involving Cit-AuNPs and silica. In both units, the discussion begins with colloidal homoaggregation results in KCl before presenting heteroaggregation data. Next, homoaggregation in EPA test water is delineated, followed by heteroaggregation in the same aqueous chemistry.

4.1 Heteroaggregation of Cit-AuNPs with Hematite

4.1.1 Hematite Homoaggregation in a 1:1 Electrolyte

Understanding the homoaggregation behavior of hematite is an important preliminary step. This establishes a baseline that can be compared to samples containing both hematite and Cit-AuNPs. It also ensures that hematite is stable in the conditions under investigation. Once stability has been illustrated, destabilization in the presence of Cit-AuNPs can be attributed to heteroaggregation.

To characterize colloidal hematite, zeta potential measurements were taken in the absence of SR-NOM, between pH 4-10 [51]. This data is presented in Figure 4-1. In acidic and neutral conditions, the hematite particles exhibited a net positive surface charge. As the pH becomes more alkaline, surface sites deprotonate which facilitates a change to increasingly negative charge. This causes the zeta potential to decrease.
Charge reversal was seen at approximately pH 9.7. At this point, the net charge of the hematite particles is neutral.

Smith found that hematite homoaggregated between pH 9.2-10.2 [51]. There was a strong correlation between hematite destabilization in alkaline water to the low net surface charge seen in Figure 4-1. The low surface charge of the hematite particles decreases the energy barrier to interaction which allows particles to homoaggregate.

Some of the experimental work described below looked at hematite and Cit-AuNPs in 1mM KCl at pH 10. A control sample containing hematite at this condition showed no destabilization, according to TR-DLS. Smith also showed that when this solution was mixed, the IEP decreased to about pH 8. A small amount of mixing in this work likely accounts for hematite particle stability at pH 10.
Figure 4-1: Zeta potential of hematite in the presence and absence of 1 mg/L SR-NOM in 1mM KCl

Figure 4-1 also reveals that the addition of 1mg/L SR-NOM changed the surface charge character of hematite. At low pH, there was a dramatic difference between the zeta potential of bare hematite and hematite with SR-NOM. The zeta potential at pH 4.1 with no organic matter was about 52 mV, while the zeta potential at pH 4.5 with SR-NOM was −79.6 mV. Hematite in 1mM KCl showed an inverse relationship between pH and zeta potential. Zeta potential remained relatively unchanged as pH was adjusted in the presence of SR-NOM. As previously mentioned,
adsorption of organic matter to colloidal materials and other types of particles is well-documented. Therefore, it is reasonable that SR-NOM imparts a negative charge on the surface of hematite particles upon adsorption.

Hematite homoaggregation in the presence of monovalent KCl was examined at pH 6 and is presented in Figure 4-2. α describes the ratio of the initial homoaggregation rate constant at some ionic strength and the diffusion limited initial homoaggregation rate constant. The CCC is reached at the KCl concentration where \( \log(\alpha) = 0 \). When no SR-NOM was present, the experimentally determined CCC was approximately 70 mM (\( \log(\text{KCl}) = -1.15 \)). The addition of SR-NOM increased the CCC to 200 mM (\( \log(\text{KCl}) = -0.70 \)). This indicates a stabilizing effect of SR-NOM on hematite in the presence of a 1:1 electrolyte such as KCl. Clearly, a much higher ionic strength is required to reach the diffusion-limited aggregation regime in the presence of SR-NOM. This agrees with previous research which demonstrated that 10mg/L DOC stabilized hematite nanoparticles in 10 mM KCl [5].
4.1.2 Influence of pH and SR-NOM on Cit-AuNP Heteroaggregation with Hematite in a 1:1 Electrolyte

Figure 4-2 demonstrates that hematite did not homoaggregate in 1mM KCl.

Additionally, 1mg/L SR-NOM stabilized the particles. As mentioned above in Chapter 3, previous work showed that Cit-AuNPs were also stable in KCl at the pH conditions
of interest. This established that aggregation of hematite colloids in the presence of Cit-AuNPs as measured by TR-DLS was attributable to heteroaggregation.

Figure 4-3 shows the extent of aggregation as a function of Cit-AuNP concentration in 1mm KCl. Data at pH 6.1 and pH 8 from the work by Smith are included for comparison [51]. Up to 1.5 mg/L Cit-AuNPs were added to hematite at pH 10. The aggregation extent remained flat at this pH and no effect of Cit-AuNP concentration was observed. This resembles the plot of hematite homoaggregation behavior at pH 10 mentioned above. Hematite and Cit-AuNPs both have a net negative charge under these conditions. Therefore, electrostatic repulsion discouraged interparticle interactions and prevented heteroaggregation.
Figure 4-3: Aggregation extent of hematite and Cit-AuNP in the presence and absence of SR-NOM

The extent of aggregation profile at pH 6 and 8 with 1mg/L SR-NOM was similar to the profile at pH 10 in the absence of SR-NOM. The system remained stable, regardless of Cit-AuNP concentration. Adding SR-NOM dramatically changed hematite surface charge behavior as seen in Figure 4-1. Moreover, prior studies have reported an increasingly negative surface charge on stable gold nanoparticles in the presence of organic matter [8]. The hematite charge reversal and enhanced charge stabilization of the Cit-AuNPs from SR-NOM adsorption prevented heteroaggregation.
Experiments discussed so far have included a maximum of 1mg/L SR-NOM. However, it is reasonable to expect the concentration of organic matter in natural systems to vary spatially and temporally [43]. Therefore, it is desirable to determine the minimum concentration of SR-NOM required to stabilize a system containing Cit-AuNPs and hematite. Figure 4-4 shows the distribution of Cit-AuNPs following exposure to hematite for 30 minutes in the presence of varying concentrations of SR-NOM. In all cases, 1.0 mg/L Cit-AuNPs were initially added.

![Figure 4-4: Filtration data for 10 mg/L hematite, 1mg/L Cit-AuNPs and 0.005-0.5 mg/L SR-NOM at pH 6](image-url)
In the presence of 0.5 mg/L SR-NOM, 78% of the Cit-AuNPs initially transferred to the sample were identified in the filtrate while approximately 21% was retained on the filter. Results were similar for the case with 0.05 mg/L SR-NOM. In this sample, about 81% of initially added gold was found in the filtrate while 16% was recovered with the retentate. The fractionation of gold in the sample with 0.005 mg/L SR-NOM deviates substantially from the others. Only 2% of the gold was recovered in the filtrate while 73% was captured on the filter. The rest was unrecovered.

When the SR-NOM concentration decreased to 0.005mg/L, less than 5% of the Cit-AuNPs were detected in the filtrate which indicates extensive attachment with hematite. The low free Cit-AuNP concentration was probably due to minor organic matter coating and associated charge reversal on the hematite particle surface, which prevented complete attachment. Therefore, heteroaggregation with hematite accounts for the high concentration of gold captured by the filter.

About 25% of the gold was not accounted for in this sample which introduces uncertainty. However, the lost gold is probably an artifact of incomplete filter digestion. The percentage of recovered iron was also relatively low. Since minimal iron was detected in the filtrate, lost iron was likely from hematite heteroaggregates retained on the filter. Incomplete re-suspension before ICP-OES analysis probably accounts for unrecovered Cit-AuNPs. In this research, samples containing Cit-AuNPs and 1mg/L SR-NOM in 1mM KCl were analyzed and filtered, with high filtrate
recoveries detected. Only about 2% of Cit-AuNPs were captured by the filter, which shows accurate filtrate measurement. Additionally, filtered samples containing Cit-AuNPs in 1mM KCl were analyzed by Smith. These results also showed nearly 100% Cit-AuNP recovery in the filtrate and negligible concentrations in the retentate [51]. This further suggests that Cit-AuNPs were accurately measured in the filtrate and that the unrecovered fraction in filtration experiments was likely associated with colloidal particles captured on the filter.

Lost gold could have been in the filtrate as unattached Cit-AuNPs. However, even if the entire fraction of unrecovered gold was unattached Cit-AuNPs, the total free Cit-AuNP concentration would still be less than 30% of the initial concentration. This is much lower than the approximately 80% free Cit-AuNPs seen with 0.05 and 0.5 mg/L SR-NOM. 0.005 mg/L SR-NOM was therefore not sufficient to prevent significant Cit-AuNP attachment to hematite.

Fractionation data is presented in Figure 4-5 which complements extent of aggregation results by quantifying Cit-AuNP attachment to hematite. The 1:1 line serves as a guide, representing conditions where Cit-AuNPs were completely stable and 100% passed the membrane filter (i.e., do not associate with hematite). Data collected from Smith at pH 6.1 and 8 without SR-NOM are also shown for comparison [51].
Figure 4-5: The concentration of Cit-AuNPs in the filtrate following filtration in 1mM KCl at several pH conditions and in the presence and absence of SR-NOM, as a function of initial Cit-AuNP concentration [51]

Samples containing 0.6 and 1.0 mg/L Cit-AuNPs were analyzed at pH 10. When 0.6 mg/L Cit-AuNPs were initially added, about 25% of the gold stayed on the filter, indicating attachment to hematite. Similarly, 29% of 1mg/L Cit-AuNPs initially in the sample heteroaggregated. These two points further affirm that as the hematite surface charge becomes more negative, fewer Cit-AuNPs interact with hematite surface sites. Although some Cit-AuNPs attached to hematite, most of them remained free in suspension. The trend also resembles the 1:1 line. As the initial Cit-AuNP
concentration increased, more unattached gold was detected. The figure also illustrates that significantly less free Cit-AuNPs are recovered at lower initial concentrations at pH 10 than at pH 6.1 or 8, which indicates fewer attachment sites are available at higher pH. At pH 10, hematite has a net negative charge but there are probably areas on the surface with positive charge due to the proximity to the IEP. Those regions can electrostatically interact with Cit-AuNPs according to principles delineated in the electrostatic patch model. This is likely responsible for the significant fraction of Cit-AuNPs attached to hematite.

At pH 6, adding 1mg/L SR-NOM significantly increased the free gold concentration relative to the case without SR-NOM. In the absence of organic matter, detectable concentrations of gold were not seen in the filtrate until the initial concentration of gold was 1.5 mg/L. When SR-NOM was added, approximately 92% of the 0.1 mg/L Cit-AuNPs initially in solution were detected in the filtrate. Experiments with Cit-AuNP concentrations up to 0.74 mg/L yielded similar results. SR-NOM coated both the hematite and Cit-AuNPs, inhibiting attachment. Including 1mg/L SR-NOM stabilized the system where extensive aggregation had been observed in its absence. It is also interesting to note that stabilization was achieved, regardless of Cit-AuNP concentration.

At pH 10, 0.5 and 1.0 mg/L SR-NOM also stabilized the colloidal system. The filtrate concentration reached nearly 95% of the initial Cit-AuNP concentration in the presence of 0.5 mg/L SR-NOM. Doubling the concentration of organic matter
increased the stable fraction of the Cit-AuNPs to 103% of the initial value. The slight increase in gold concentration was probably due to experimental error. Increasing the concentration of SR-NOM resulted in a minor increase in free Cit-AuNP concentration in both samples, but negligible or very low Cit-AuNP mass was detected with hematite on the filter. This indicates that nearly complete Cit-AuNP stabilization was achieved with only 0.5 mg/L SR-NOM, which is relatively low for a natural system.

The extents of aggregation for samples containing 0.005-1.0 mg/L SR-NOM at pH 6 are displayed in Figure 4-6. The system was stable with respect to aggregation at SR-NOM concentrations above 0.05 mg/L, as evidenced by constant values of \(D_{h,30}/D_{h,0}\). However, when the SR-NOM concentration was decreased to 0.01 mg/L SR-NOM, Cit-AuNPs and hematite rapidly aggregated. Further reduction in SR-NOM induced a slightly greater extent of aggregation, similar to that of hematite and Cit-AuNPs with no organic matter, as shown by Smith [51]. The aggregation extent with no SR-NOM and 0.005 mg/L SR-NOM was 4.76 and 4.01, respectively. This illustrates that 0.005 mg/L SR-NOM doesn’t enhance or reduce stability in 1mM KCl at pH 6.
Figure 4-6: Filtration and TR-DLS data for 0.6 mg/L Cit-AuNPs and 10 mg/L hematite as a function of SR-NOM content.

The extent of aggregation of suspensions at pH 10 is also displayed in Figure 4-6. These experiments were also performed with 0.6 mg/L Cit-AuNPs. No change in particle size was detected with the addition of 0.1-1.0 mg/L SR-NOM, which is consistent with the stabilization observed in the absence of SR-NOM. The free Cit-AuNP concentrations with 0.5 and 1.0 mg/L SR-NOM at pH 10 are also shown here.
In both cases, the free Cit-AuNP concentration is approximately 100% of the initial concentration.

With up to 0.005 mg/L SR-NOM at pH 6, particles heteroaggregated to approximately four times the initial hematite particle size. The high aggregation extent closely agrees with filtration data where trace amounts of free Cit-AuNPs remained in solution. Higher concentrations of SR-NOM prevented heteroaggregation as observed by TR-DLS and resulted in greater free Cit-AuNP concentrations after fractionation.

In the 0.5-1.0 mg/L SR-NOM range, a higher percentage of Cit-AuNPs remain free at pH 10 than at pH 6. The plot demonstrates that the extent of aggregation and free Cit-AuNPs concentration are inversely related at pH 6. Extensive aggregation and lower concentrations of free Cit-AuNPs are correlated with lower SR-NOM concentrations. This trend is also seen at pH 10 with 1mg/L SR-NOM. Figure 4-6 demonstrates that low concentrations of SR-NOM down to approximately 0.05mg/L as DOC stabilize Cit-AuNPs and hematite in a relatively simple solution with a single monovalent salt. Because this concentration is much lower than that typically found in the environment, these results indicate that Cit-AuNPs in a natural aqueous system would mostly be in free suspension, rather than heteroaggregated with colloidal particles.
4.1.3 Cit-AuNP Homoaggregation in Standard Test Water

Cit-AuNP homoaggregation was also studied in EPA standard test water. Figure 4-7 shows that the extent of aggregation for the Cit-AuNPs is directly related to the test water ionic strength and hardness. The maximum aggregation extent is achieved in very hard water and tends to decrease as ionic strength and hardness also decrease.

Figure 4-7: Extent of Cit-AuNP homoaggregation in standard test water of varying hardness
The same experiment was carried out with the addition of 1mg/L SR-NOM. SR-NOM decreased the extent of aggregation in all cases which is in agreement with other experiments from this study. Interestingly, the largest decrease in aggregation was seen in moderate strength water, while Cit-AuNPs in very hard and very soft water were relatively unaffected. In the latter two conditions, homoaggregation extent with 1mg/L SR-NOM was approximately 90% of the extent seen without SR-NOM. However, in water hardness conditions of 41.9-167.6 mg/L as CaCO₃, aggregation decreased by 40-60%. The divalent cation concentrations in each dilution could explain the weaker stabilization effects of SR-NOM seen in hard and very hard water.

The greater concentration of Ca²⁺ and Mg²⁺ ions may have facilitated bridging between some of the SR-NOM coated Cit-AuNP particles which resulted in a net stabilization effect that is lower than in moderately hard water conditions. Stankus et al. showed that Cit-AuNPs stabilization in the presence of 5mg/L SRHA could be predicted from Mg²⁺ and Ca²⁺ concentrations. Generally, aggregation was enhanced at ionic strengths greater than 50mM which is much higher than in any of the test water dilutions used here. However, slower initial aggregation rates were detected at the 10mM which is the lowest Ca²⁺ the researchers used in that experiment [8]. It should also be mentioned that the 50mM ionic strength required for SRHA to enhance aggregation was in the presence of a single divalent electrolyte whereas EPA test water contains monovalent electrolytes that do not undergo interparticle bridging with organic matter. Since the test water conditions are more dilute and contain monovalent
species, the data presented by Stankus agrees with the stabilization from SR-NOM shown in Figure 4-7.

Cit-AuNPs in very soft and very hard water were also filtered and analyzed using ICP-OES. The TR-DLS results indicated that Cit-AuNPs in very soft water should largely pass through the filtration apparatus. With an aggregation extent of about 2, most of the Cit-AuNPs were expected to be recovered in the filtrate. In very hard water, the Cit-AuNPs aggregated to about 10 times their original diameter. This suggested that Cit-AuNPs would be mostly recovered in the sample retentate. Figure 4-8 expresses the fate of 1mg/L Cit-AuNPs suspended with hematite in very soft and very hard water. In very hard water, about 90% of the gold placed in the sample was recovered in the retentate, while roughly 1% was detected in the filtrate. The remainder was unaccounted for.
Figure 4-8: Fractionation of 1mg/L Cit-AuNPs in very hard and very soft standard test water.

Pervasive aggregation in very hard water is most likely due to double layer compression. It may be recalled from Table 3-1 the ionic strength in very hard water is about 18mM. Although this is well below the experimentally determined CCC for Cit-AuNPs in KCl, the standard test water contains several multivalent species, including CaCl$_2$. McDowell found Cit-AuNPs aggregate according to the diffusion-limited regime in 2.28mM CaCl$_2$ [42]. Similar results were also reported by Liu et al.[43]. The
contribution to ionic strength from Ca\(^{2+}\) alone is about 2.67 mM so the aggregation behavior seen here is expected.

In very soft water, the Cit-AuNP recovery by the filter dropped to 9%. About 76\% of the 1mg/L Cit-AuNPs were detected in the filtrate and the remaining 15\% was unrecovered. The substantial drop in Cit-AuNPs captured by the filter is also in agreement with TR-DLS results. However, it is interesting that nearly 10\% of the gold was found on the filter when the effective particle diameter after 30 minutes as determined by TR-DLS was 52.7 nm. This is well under the 80 nm cutoff on the filter. It is possible that although the average particle size is less than 80 nm, there may have been a fraction of larger homoaggregates also present that were removed by the filter.

Cit-AuNPs in moderate hardness conditions were not analyzed by filtration. A logical hypothesis is that as the water hardness is increased, a greater fraction of the initial Cit-AuNPs would be recovered on the filter.

In very soft water, the total ionic strength is approximately 0.6 mM. The contribution from divalent Ca\(^{2+}\) and Mg\(^{2+}\) is only about 0.1 mM which is more than one order of magnitude lower than the CCC concentration. Very soft water is also much more dilute than the 56 mM KCl required to reach its CCC. Therefore, limited homoaggregation with an extent of approximately 2 in very soft water is not predicted from the CCC of Cit-AuNPs for individual monovalent and divalent electrolytes. It remains unclear what caused this behavior.
4.1.4 Influence of SR-NOM on Hematite Homoaggregation in Standard Test Water

Systems with a single monovalent salt like KCl were used to investigate aggregation behavior in a relatively simple chemical matrix. On the other hand, natural water bodies are typically much more complicated and contain a variety of electrolytes and minerals. The presence of these other constituents can have a substantial impact on both homo- and heteroaggregation. One of these components is CaCl$_2$, which is also a component of EPA standard water. To more accurately assess hematite homoaggregation in a natural system, analysis was conducted in the presence of CaCl$_2$ (Figure 4-9).

When CaCl$_2$ was used, the CCC decreased from 70 to 20 mM. This conflicts with the Schulze-Hardy rule which predicts that the concentration of a divalent salt required to reach the CCC is 100 times lower than for a monovalent salt [35]. Deviations from the Schulze-Hardy rule have been reported for metal oxide particles. Chen found that the ratio of NaCl to CaCl$_2$ necessary for hematite nanoparticles to aggregate in the diffusion-limited regime was only 12.5. The authors attribute this to the relatively low zeta potential at the tested conditions [38]. However, pH conditions where bare hematite was examined were much more alkaline (~pH 12.2) than in this work. Interestingly, the same investigation found that bare hematite and hematite pre-coated with SRHA had similar CCCs in CaCl$_2$ solution. However, enhanced stability was observed in NaCl solution [38]. The results presented here are pretty similar to
those of Chen. In KCl solution, SR-NOM stabilized the hematite and increased the CCC from 70 to 200 mM. When only CaCl$_2$ was present, SR-NOM helped facilitate homoaggregation and the CCC decreased.

When SR-NOM was introduced, log ($\alpha$) increased above 0, indicating that aggregation was enhanced by the presence of SR-NOM and high concentrations of CaCl$_2$. The increased aggregation was due to interparticle bridging between the SR-NOM and Ca$^{2+}$ cations which has been noted elsewhere in the literature [5, 8, 38].

Figure 4-9: CCC results for hematite at pH 6, in the presence of CaCl$_2$
Homoaggregation of the hematite colloids was then investigated in the EPA test waters. Extents of aggregation for hematite in the presence and absence of 1mg/L SR-NOM are shown in Figure 4-10. In the absence of SR-NOM, hematite homoaggregates slightly, to an extent of about 1.25 in very soft water, but to a much greater extent in the other four classes of test water. Hematite particles were homoaggregating at a reaction-limited aggregation rate in very soft water. The salt content in soft-very hard water allowed hematite to homoaggregate in the diffusion-limited regime. As the salt concentration increased, the extent of aggregation and the initial slope remained fairly constant.

Table 3-1 shows that the ionic strength in standard test water is much lower than the CCC in KCl and CaCl₂ of 70 mM and 20mM, respectively. Figure 4-9 shows that homoaggregation was observed at Ca²⁺ concentrations as low as about 1mM. Salt content alone does not account for the homoaggregation in very soft water. However, the test water pH also increases with hardness. Higher pH conditions decreases the hematite zeta potential and lowers electrostatic repulsion. The lower surface charge and salt content could induce reaction-limited homoaggregation.
When 1mg/L SR-NOM was added, hematite remained stable throughout the testing period. At this concentration, SR-NOM coated the hematite particles sufficiently to inhibit aggregation and did not appear to participate in cation bridging with calcium or magnesium. The maximum calcium concentration in EPA test water is about 1.3mM. According to Figure 4-9, very little homoaggregation was detected at roughly 2mM CaCl₂ at pH 6, so complete stabilization is predicted at the lower concentrations present in more dilute test water. In their report on nanoparticle
stability, Zhang et al. examined homoaggregation of 10mg/L hematite coated with 4mg/L SR-NOM. When 2-16 meq/L Ca$^{2+}$ were also added, enhanced aggregation was seen over a 1-hour period [5]. These results suggest that hematite might homoaggregate to a greater extent in the presence of SR-NOM and test water, which conflicts with results in Figure 4-10.

The stabilization from SR-NOM might be due to the lower SR-NOM concentration. 1mg/L was not a sufficient amount of organic matter to participate in interparticle bridging. These results highlight the fact that NOM’s tendency to promote aggregation or stability in standard test water is difficult to predict based on studies conducted in only one electrolyte.

4.1.5 Heteroaggregation of Cit-AuNPs with Hematite in Standard Test Water

Due to the extensive homoaggregation of hematite and Cit-AuNPs in standard test waters without organic matter, heteroaggregation was only examined by TR-DLS in the presence of SR-NOM. Figure 4-11 depicts aggregation extents in standard test water containing 1mg/L Cit-AuNPs, SR-NOM and hematite. In very soft and soft water, the aggregation extent remained at approximately 1. In moderately hard and hard water, aggregation was seen but the extent increases by less than 10% in both samples. Much greater aggregation occurred in very hard water. Figure 4-10 indicates
that hematite in the absence of Cit-AuNPs was stable in these conditions while Cit-AuNPs homoaggregated in the presence of SR-NOM (Figure 4-7). This indicates that the aggregation seen in moderately hard to very hard water was a result of Cit-AuNP homoaggregation.

Figure 4-11: Hematite and 1mg/L Cit-AuNP aggregation extents in standard test water with 1mg/L SR-NOM
However, evidence of Cit-AuNP heteroaggregation is shown in Figure 4-12 A) and B). The measured particle size over time is plotted for 1mg/L Cit-AuNPs, 10mg/L hematite, and a mixed system containing both particle species in hard and very hard water. 1mg/L SR-NOM was also included in both cases.

Figure 4-12: Hydrodynamic diameter as a function of time for hematite, 1mg/L Cit-AuNPs and hematite + Cit-AuNPs with 1mg/L SR-NOM in A) hard water and B) very hard water.
As discussed above, hematite particles were stable in both water hardness conditions. The plot of particle size vs. time remained essentially flat through the experiment. Figure 4-12 A) illustrates that the Cit-AuNPs initially homoaggregated very rapidly. However, in the sample containing both particle species, the hydrodynamic diameter growth was much slower. The fast Cit-AuNP homoaggregation was not immediately detected by the instrument so the average particle size stayed flat at the beginning of the experiment. The measured particle size in the binary system slowly increased with further Cit-AuNP attachment and homoaggregate growth.

Cit-AuNP homoaggregation does not completely explain the particle growth seen in the binary system. Towards the end of the experiment, the average particle size surpassed that of the system containing only hematite. When alone, Cit-AuNPs homoaggregated to nearly 130 nm, which is still noticeably smaller than the hematite particles. If Cit-AuNPs were only homoaggregating in the presence of hematite, the average particle size should have decreased due to smaller homoaggregates. Because particle growth is more extensive in the system containing hematite and Cit-AuNPs than just hematite, Cit-AuNPs were both homoaggregating and heteroaggregating with hematite. However, Cit-AuNP attachment to hematite is not clear from TR-DLS results.

Figure 4-12 B) illustrates that Cit-AuNPs initially homoaggregated nearly five times faster in very hard water than in hard water. At the start of the experiment with
both particle species, the average particle size only reflected that of the hematite particles. The Cit-AuNP homoaggregates were initially smaller than the hematite which briefly decreased the average particle size in the sample. The average particle size then increased linearly with time. At times greater than about 10 minutes, the plot of Cit-AuNP homoaggregate growth is qualitatively similar to that of Cit-AuNPs + hematite. Although these plots have similar growth rates, the average particle size of the binary system is clearly greater than that of only Cit-AuNPs. This is further evidence that Cit-AuNPs heteroaggregated with hematite in hard and very hard water with SR-NOM.

There is also an indication that Cit-AuNPs and hematite heteroaggregated in standard water with low ionic strength and no SR-NOM. Figure 4-13 displays filtration results from samples in very soft and very hard water. In very soft conditions and no hematite, about 75% of the gold was recovered in the filtrate while less than 10% was retained by the filter. This contrasts with samples containing hematite where 70% of the gold was captured by the filter and a trivial amount passed through the filter. The seven-fold increase in gold retained by the filter suggests that Cit-AuNPs were attached to hematite and heteroaggregating under these conditions. Additionally, the paucity of gold passing through the filter means that nearly all of the gold was successfully attaching to hematite.

There was a loss of nearly 30% of the initial gold in the mixed system, which makes a more quantitative comparison difficult. Again, the most probable explanation...
is that Cit-AuNPs associated with the hematite particles on the filter were not re-suspended after filtration. The filter was not completely digested, so it is possible that the aqua regia mixture did not sufficiently remove and dissolve captured heteroaggregates.

![Figure 4-13: Cit-AuNP homoaggregation and heteroaggregation with hematite in EPA test water, with no SR-NOM](image)

The results in very hard water also suggest that Cit-AuNPs were homoaggregating, rather than attaching to hematite. Regardless of hematite content, less than 1% of the Cit-AuNPs were recovered in the filtrate. There is also a
substantial difference in the heteroaggregated Cit-AuNP concentration. The concentration of Cit-AuNPs captured on the filter was 10% higher when no hematite colloids were present. Unfortunately, total Cit-AuNP recovery was 10% lower in samples with hematite. As previously mentioned, unrecovered Cit-AuNPs in fractionation experiments were probably associated with un-digested hematite. Assuming the unrecovered fraction of Cit-AuNPs was attached to the filter in both cases, the free Cit-AuNP concentrations were approximately equal. This does not indicate Cit-AuNPs heteroaggregated with hematite in very hard water and no SR-NOM.

Fractionation data in Figure 4-13 illustrates that in relatively dilute standard water conditions, Cit-AuNPs heteroaggregate considerably with hematite, in the absence of SR-NOM. More extensive heteroaggregation is expected in higher hardness conditions but the data in very hard water does not confirm this. TR-DLS results discussed above indicate that heteroaggregation occurred in these aqueous conditions when 1mg/L SR-NOM is added. However, Cit-AuNP attachment to hematite was not quantified.
4.2 Heteroaggregation of Cit-AuNPs with Silica

4.2.1 Silica Homoaggregation in a 1:1 Electrolyte

Silica homoaggregation was examined in KCl solution at pH 6, with results shown in Figure 4-14. The calculated CCC was 280 mM. This is very similar to the CCC determined for 25 nm silica nanoparticles in NaCl [53]. The data also indicates that silica is more stable in KCl than an equivalent concentration of hematite at the same pH. Silica colloids did not homoaggregate in 1 mM KCl and the particles were stable in the absence of Cit-AuNPs.

![Figure 4-14: Plot of log(α) vs. KCl concentration for silica colloids at pH 6. Error bars represent 95% confidence intervals. In several instances, calculated negative error bars were undefined.](image-url)
Heteroaggregation with silica was not detected with TR-DLS at pH 6. Figure 4-15 shows that in the absence of SR-NOM, up to 1.0 mg/L Cit-AuNPs did not induce destabilization. The extent of aggregation remained at about 1, regardless of Cit-AuNP concentration. Silica has a net negative surface charge at pH 6. Since the Cit-AuNPs are also negatively charged, it is likely that electrostatic repulsion was largely responsible for the lack of heteroaggregation.

Aggregation was also analyzed in the presence of 1mg/L SR-NOM. The data in Figure 4-15 demonstrates that organic matter had no observable effect on heteroaggregation. With up to 1mg/L Cit-AuNPs, no change in average particle size was detected. The system was already stable without the steric and electrostatic stabilization effects that are commonly seen with SR-NOM.
Several studies have reported that organic matter has limited interactions with silica. Abe investigated the homoaggregation of 150 nm silica colloids with different salts and organic matter sources. The authors found that fulvic acids had no effect on attachment efficiency, even in the presence of a divalent salt [55]. It has also been suggested that silica has a very low adsorption affinity for natural organic matter [5]. Instead, the SR-NOM adsorbed to the Cit-AuNPs preferentially, further enhancing stabilization.

Figure 4-15: Aggregation profiles for Cit-AuNPs with silica in 1mM KCl at pH 6.
Heteroaggregation of Cit-AuNPs and silica was also examined through the lens of filtration experiments in the presence and absence of SR-NOM. Figure 4-16 summarizes these results. In the absence of SR-NOM, 48% of the Cit-AuNPs were detected in the filtrate. About 21% was associated with silica and the remainder was unrecovered. The addition of SR-NOM substantially increased the concentration of free Cit-AuNPs. 82% of Cit-AuNPs remained free in suspension and about 4% was associated with silica. The percentage of Cit-AuNPs associated with silica in the presence of SR-NOM is approximate because it is below the least concentrated standard used.
Figure 4-16: Distribution of Cit-AuNPs in filtered samples containing silica and 1mg/L Cit-AuNPs at pH 6 in 1mM KCl

The samples with no SR-NOM suggest that a significant fraction of Cit-AuNPs associated with silica colloids. It is possible that the Cit-AuNPs are chemically adsorbing to the surface of silica particles even though both species are negatively charged. The citrate capping agent on the Cit-AuNPs could have chemically reacted with the silanol surface of silica [53]. At pH 6, most of these silanol groups are deprotonated and carry a negative charge. Citrate also has relatively low pka values at 3.1, 4.7 and 6.3. Acidic groups on citrate that are characterized by the two lower pkas
are also completely deprotonated at pH 6. However, roughly half of the sites with a pka of 6.3 remain protonated. Those protonated sites could interact with the negatively charged silanol groups on the silica particle surface, resulting in minor heteroaggregation.

However, a precise estimate of gold attachment is not clear from Figure 4-16. Approximately 20% of the gold initially added was recovered with silica but about 30% of the gold was also unaccounted for. Moreover, silica recovery was inconsistent and generally poor. For these samples, the average silica mass recovery was about 40%. In control samples containing just silica and no Cit-AuNPs, mass recoveries were lower than 40%. However, only about 2% of the silica initially in the samples passed through the filter. Although most of the silica was not detected after fractionation, only a very small fraction passed through the filter which indicates that nearly all of the heteroaggregated gold was held on the filter.

4.2.2 Silica Homoaggregation in Standard Test Water

Silica’s homoaggregation behavior in standard test water is quite different than that of hematite. Figure 4-17 shows that silica particles remained stable in all water hardness dilutions. 1mg/L SR-NOM did not change this behavior. Surprisingly, all samples containing silica showed small decreases in particle diameter. Measured
particle size decreased by an average of 8 nm. It is unclear why the diameter of silica decreased in standard water.

Zhang et al. also showed that the presence of SR-NOM and up to 8mM Ca\textsuperscript{2+} cations in solution had no appreciable effect on zeta potential or particle size of silica nanoparticles. The authors found that silica’s adsorption capacity for SR-NOM was extremely low compared to the other examined metal oxide particles. Because silica had minimal interactions with organic matter, interparticle bridging was not observed. DLVO modelling was also performed to complement their experimental data. The results showed that Ca\textsuperscript{2+} concentrations much greater than those found in standard test water could not sufficiently reduce the energy barrier to homoaggregation in the presence of SR-NOM [5]. These findings agree with the stabilization seen in standard test water.
Silica’s stability in Figure 4-17 is also in agreement with the CCC data presented above in Figure 4-14. The 280 mM CCC is much greater than the ionic strength in any of the test water dilutions. Additionally, Abe reported the CCC for silica in CaCl$_2$ as about 20 mM at pH 5 [55]. This is also much higher than the divalent cation concentration in the EPA test water. The authors reported slow, reaction-limited homoaggregation at Ca$^{2+}$ concentrations as low as 1mM. This is roughly equal to the Ca$^{2+}$ concentration in very hard water. Homoaggregation was not seen in standard test water because of the elevated pH and more negative surface
charge which enhances stability. Moreover, the electrolyte concentration in even the strongest dilution of standard water was not high enough to sufficiently compress the EDL on silica particles.

4.2.3 Influence of SR-NOM and Ionic Strength on Cit-AuNP Heteroaggregation with Silica in Standard Test Water

Samples containing silica and 1mg/L Cit-AuNPs in standard test water were analyzed, with results displayed in Figure 4-18. A set of similar samples also included 1mg/L SR-NOM. Without SR-NOM, aggregation was only observed in hard and very hard water. Even at these relatively high ionic strength conditions, the maximum extent of aggregation was only about 1.3. In lower ionic strength samples, the effective particle diameter in the system actually decreased slightly over the experimental period. The inclusion of SR-NOM did not affect aggregation in these conditions. In hard and very hard water, SR-NOM impaired aggregation.
Cit-AuNPs homoaggregated in all of the tested hardness conditions. This attachment likely accounts for the decreasing particle size of the mixed system containing both particle species. Figure 4-19 shows the change in hydrodynamic diameter of Cit-AuNPs, silica and Cit-AuNPs + silica in very hard water A) without SR-NOM and B) with SR-NOM.

When SR-NOM was absent, Cit-AuNPs rapidly homoaggregated. At the end of the trial, the measured particle size had increased by a factor of about 10. Oddly, the silica particle size decreased for the first half of the experiment. This might have been
caused by an erroneous measurement of initial size or a small number of slightly larger silica colloids. However, as the run continued, the particle size stayed constant at about 140 nm, which is approximately equal to the measured particle diameter in the stock solution. In the combined system with both species, the average particle size immediately decreased from about 150 to 110 nm. Particle diameter increased after this and followed a pattern similar to the Cit-AuNPs alone.
Figure 4-19: Hydrodynamic diameter as a function of time for Cit-AuNPs, silica colloids, and Cit-AuNPs+ silica in very hard water A) without SR-NOM and B) with 1mg/L SR-NOM
The inclusion of SR-NOM slightly decreased the extent of homoaggregation for Cit-AuNPs, alone. Silica remained stable at about 140 nm. The sudden decrease in particle diameter and ensuing increase was driven by the homoaggregation of Cit-AuNPs, rather than heteroaggregation. In both scenarios depicted in Figure 4-19, the plot of Cit-AuNP + silica particle size after approximately five minutes is consistently higher than that of silica, and lower than that of just Cit-AuNPs. In the binary system, Cit-AuNP homoaggregates began to dominate the scattered light intensity at this time. This resulted in an increased calculated average particle size above the size of primary silica particles. Heteroaggregation might have also occurred to a small extent. The filtration study conducted in 1mM KCl at pH 6 illustrates that approximately 20% of the Cit-AuNPs attached to silica in the absence of SR-NOM. Therefore, it is reasonable to expect that some fraction of the Cit-AuNPs were also heteroaggregating in a system with much higher ionic strength and the inclusion of divalent cations. However, Cit-AuNP homoaggregation was the more dominant process.

4.3 Summary of Findings

Understanding the fate and transport of nanoparticles is an important prerequisite for predicting their environmental toxicity. It is also widely accepted that aggregation will strongly influence this process. This study has examined how Cit-
AuNPs heteroaggregated with two colloids that are expected to closely model those that are commonly found in environmental systems.

In relatively simple systems with a single monovalent electrolyte and no organic matter, the pH and its effects on surface charge dictated how extensively nanoparticles attached to the model colloid. Greater attachment was observed when the Cit-AuNPs and colloid had oppositely charged surfaces. Relatively minor heteroaggregation occurred when the two particle species had like surface charges. In the presence of organic matter, the pH was less influential. 1mg/L SR-NOM had a strong stabilizing effect which greatly decreased Cit-AuNP heteroaggregation with both colloids. Since organic matter is ubiquitous in environmental systems, these results imply that nanoparticles would be largely stabilized and heteroaggregation prevented.

A similar trend was seen in the EPA standard test water samples which better represent natural aqueous chemistry. When no organic matter was introduced, heteroaggregation with hematite was detected at relatively low ionic strength but even at higher ionic strengths, 1mg/L SR-NOM prevented heteroaggregation with both colloids, regardless of surface charge. Cit-AuNPs homoaggregated in all hardness conditions, and the presence of SR-NOM had a minor stabilizing effect on this process. Although rapid and very extensive homoaggregation was observed, low nanoparticle concentrations expected in the environment would decrease the importance of this process on fate and transport. In natural water, the adsorption of
organic matter to colloidal particles will impart a net negative charge which electrostatically repels nanoparticles with like charge. Evidence presented in this work shows that organic matter adsorption would prevent heteroaggregation with colloidal particles.
5. Conclusions

This work builds on earlier heteroaggregation studies by extending analytical methods recently developed by Smith [51] to more complicated systems containing natural organic matter, varying ionic composition, and different model colloids. The analysis of the results described above leads to several conclusions about the heteroaggregation of Cit-AuNPs with model colloids and their environmental fate and transport.

Evidence was presented which indicates that when ionic strength was controlled by a single monovalent salt, Cit-AuNP heteroaggregation was dominated by pH in the absence of organic matter. The influence of pH on colloid surface charge could be used to predict the extent of Cit-AuNP attachment. However, the presence of organic matter dramatically reduced Cit-AuNP attachment with both colloids and the role of pH on heteroaggregation was diminished. Although some heteroaggregation was detected in these testing conditions, the concentration of organic matter in the environment is expected to be much greater than that of Cit-AuNPs and other nanoparticles [2, 6, 43]. The concentration of organic matter found in the environment is also typically higher than the 1mg/L SR-NOM examined in this work. Therefore, greater nanoparticle stabilization is predicted in natural systems which would facilitate increased environmental transport. Those nanoparticles will likely remain in solution
and be widely dispersed. As a result, susceptible organisms living farther downstream could be more at risk of toxic effects than those near the source of contamination.

In more complex environments containing divalent cations, Cit-AuNPs could heteroaggregate more extensively with colloids of both like and unlike surface charge if minimal natural organic matter is present. However, experimental results showed that only 1mg/L SR-NOM in a natural aqueous matrix will greatly reduce the extent of Cit-AuNP heteroaggregation by coating both nanoparticles and colloids. Moreover, colloidal surface charge relative to that of the nanoparticle is expected to have a limited impact on heteroaggregation. This will likely result in increased transport away from the source of nanoparticle discharge, while also affecting toxicity and chemical reactivity [2]. The following points summarize experimental results and suggest possible topics for future nanoparticle fate and transport studies.

- Cit-AuNP association with colloid particles in 1mM KCl is pH-dependent when the colloid’s IEP occurs at an environmentally relevant pH. In an environmental setting, more extensive association is likely when the colloid and nanoparticle of interest are oppositely charged.

- Silica and Cit-AuNPs have minimal interactions in 1mM KCl at pH 6. This trend is also expected to be seen across a wider pH range because of silica’s negative surface charge and low IEP.
• SR-NOM stabilized nanoparticle-colloid systems in 1mM KCl, suggesting that organic matter prevents these species from heteroaggregating in low ionic strength environments dominated by a monovalent salt.

• The presence of divalent cations including calcium and magnesium enhanced Cit-AuNP homoaggregation in EPA test waters and minimized the stabilization effects of SR-NOM. In a system containing monovalent and divalent cations, heteroaggregation of Cit-AuNPs with a colloid of similar surface charge was not observed. However, there is evidence of attachment to oppositely charged hematite and more extensive heteroaggregation is expected with increasing ionic strength in natural systems.

• The addition of SR-NOM prevented heteroaggregation with both model colloids. Water hardness and ionic strength was not sufficient to overcome the electrostatic and steric stabilization effects of the organic matter.

The scope of this research can be broadened in several directions. There is evidence available that demonstrates how the capping agent applied to a nanoparticle influences chemical reactivity, toxicity and transport. Therefore, it is reasonable and necessary to investigate heteroaggregation behavior of nanoparticles with capping agents besides citrate [8, 14, 23, 42]. Gold nanoparticles are an advantageous material to use for this because there are a wide variety of compatible capping agents [31, 56]. Altering the capping agent can introduce different surface charge character which has
important consequences on heteroaggregation, as shown in this work. Capping agents can also stabilize nanoparticles through steric effects which may react to changes in aqueous chemistry differently than those exerting electrostatic repulsion [8, 57].

Further, heteroaggregation research utilizing other organic matter models may allow for more accurate nanoparticle risk assessment in environmental systems. Other well-characterized substances besides SR-NOM should be tested to more confidently illustrate the effects of organic matter on heteroaggregation. This work showed that up to 1mg/L SR-NOM stabilized solutions containing Cit-AuNPs and two model colloids. Additional research could investigate whether higher concentrations of organic matter also enhance stabilization or promote heteroaggregation through interparticle bridging.

The EPA test water used in this research contains relevant electrolytes at concentrations likely to be encountered in the environment. However, future investigations may examine a range of salt concentrations and species that model specific surface waters. Additionally, other model colloids besides hematite and silica could be used to further analyze heteroaggregation behavior with nanoparticles.
Appendix

Low Concentrations of SR-NOM Destabilize Hematite Colloids

At 0.025 mg/L SR-NOM, the hematite particles were destabilized and rapidly formed homoaggregates in 1mM KCl. Aggregation was also seen at 0.010 mg/L SR-NOM but the extent of aggregation and initial aggregation rate substantially decreased. As the concentration of SR-NOM was lowered below 0.010 mg/L, aggregation ceased and the system remained stable. This shows that between 0.010 and 0.025 mg/L SR-NOM partially coated hematite particles such that the positive surface charge of the hematite is sufficiently reduced for particles to overcome the energy barrier to attachment. SR-NOM may have also neutralized sites with positive surface charge which decreased electrostatic repulsion or induced charge reversal that induced homoaggregation according to the electrostatic patch model. Future experiments at these conditions using zeta potential analysis could determine the appropriate mechanism.
Figure A-1: Hematite homoaggregation in the presence of ≤ 0.5 mg/L SR-NOM

TEM Imaging of Hematite and Cit-AuNP Heteroaggregates

A sample containing 10mg/L hematite, 0.6mg/L Cit-AuNPs and 1mM KCl at approximately pH 5.5 was examined using TEM (FEI Titan 80-200 TEM/STEM). The image below shows a cluster of heteroaggregates. A single, oblong hematite particle was measured with a long edge length of about 117.04 nm and a 76.42 nm short edge length. Measured Cit-AuNP diameters in this image are 12.81 and 13.69 nm which closely agrees with the manufacturer’s reported particle size.
Figure A-2: TEM image of Cit-AuNP and hematite heteroaggregates

UV-Vis Determination of Cleaned Cit-AuNP Stock Solutions

Cit-AuNPs required cleaning using centrifugation to remove excess citrate from solution prior to heteroaggregation experiments with hematite. 8 ml aliquots were removed from the purchased stock solution for a single cleaning run. Following centrifugation and re-suspension, samples were diluted and analyzed using UV-Visible spectroscopy. A calibration curve using three dilutions at 1, 2 and 3 mg/L from the original stock solution was also constructed to relate measured absorbance at 517nm in the cleaned stock solution to Cit-AuNP concentration.
Figure A-3: The calibration curve for cleaned Cit-AuNPs uses the absorbance at a wavelength of 517 nm.

Particle Size Distribution of Hematite

10 mg/L hematite in 1mM KCl was analyzed using nanoparticle tracking analysis (Nanosight, NS500). Triplicate measurements were made with 1-minute recording intervals. Particle size distributions up to 2 microns and particle concentrations were acquired. Figure A-4 shows the average cumulative particle size distribution for the triplicate samples.
Particle Size Distribution of Hematite and Cit-AuNPs in 1mM KCl

Nanoparticle tracking analysis (NTA) was also utilized to examine samples containing Cit-AuNPs and 10mg/L hematite in 1mM KCl at ambient pH. Several measurements were taken over a 30-minute period. Figure A-5 shows the particle number distribution where 0.6 mg/L Cit-AuNPs were initially introduced into the sample. Over time, the total area under the curve decreased, indicating that the total number of particles also decreased as Cit-AuNPs heteroaggregated with hematite. The
curves at t=19 and 30 minutes are also much flatter than that at t=2 minutes. Figure A-5 illustrates that the size distribution of particles widens over time.

Additionally, the average particle size also increases. Most particles were about 100 nm in diameter after two minutes but a multi-modal distribution was detected as Cit-AuNPs heteroaggregated with hematite. It should also be pointed out that the number distribution changed drastically between t=2 and t = 19 minutes but the decrease is total particles and increase in particle diameter between 19 and 30 minutes was relatively minor. This is reasonable because the heteroaggregation rate is a function of both hematite and Cit-AuNP particle concentrations. As they are consumed, fewer particles remain to participate in heteroaggregation and the rate of change in the number distribution decreases.
Figure A-5: Cumulative number concentration data for 0.6 mg/L Cit-AuNPs and 10 mg/L hematite in 1mM KCl

The same qualitative trend was seen when the concentration of Cit-AuNPs was increased from 0.6 to 1.5 mg/L. After 12 minutes, the number distribution of particles had flattened considerably and the average particle diameter increased.
Figure A-6: Number distribution for 1.5 mg/L Cit-AuNPs and 10 mg/L hematite in 1mM KCl, over a 30-minute period

Acid- Digestion Procedure Adjustments for ICP Analysis

Prior to analysis via ICP-OES, an acid mixture of concentrated HCl and HNO₃ was used to digest Cit-AuNPs and colloids. Recoveries of both iron and gold were significantly lower than desired. This was especially for true for iron. In several experiments, the amount of iron recovered on the filter was roughly half of the expected mass in control samples. As previously mentioned, it is believed that the material recovered on the filter was not being completely re-suspended. To increase
the recovered fraction of Cit-AuNPs and hematite, the digestion procedure was modified. Other researchers have utilized heat to enhance digestion prior to ICP analysis [58]. Following filtration, samples in polypropylene centrifuge tubes were heated to 50°C using a VWR Digital 2 Block Heater (VWR International, Radnor, PA). Samples were tightly capped to prevent evaporation and were allowed to sit overnight. The temperature was increased to 110°C for 10 minutes the following day before allowing the samples to cool. Iron recovery from samples was consistently higher, with roughly 80% of the iron mass detected in controls. Although this is still significantly less than complete recovery, it shows a reasonable improvement in the iron mass balance. Future fractionation experiments should build on this procedural modification. Heat may be applied in a different manner (increased heat duration, or temperature) to achieve higher mass recoveries.
References


