Effect Of Environmental Factors On The Aggregation Behavior Of Silver Nanoparticles In The Aquatic Environment

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EFFECT OF ENVIRONMENTAL FACTORS ON THE AGGREGATION BEHAVIOR OF SILVER NANOPARTICLES IN THE AQUATIC ENVIRONMENT

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DEDICATION

This dissertation research and doctoral degree is dedicated to my beloved parents and family for their endless support and encouragement. To my husband for his love, constant support and help during my PhD, and to my friends.
ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Mohammed Baalousha, whose expertise, guidance and support made a significant contribution to the completion of this study. I would like to acknowledge my supportive dissertation committee members Dr. Jamie Lead, Dr. Alan Decho, and Dr. John Ferry whose advice and guidance were invaluable. Your time and efforts are greatly appreciated. Special thanks to Dr. Geoff Scott for his support during my PhD. I am grateful to our Center Coordinator, Elizabeth Caulder and my colleagues for continued support, encouragement and collaboration. The author thanks the SmartState Center for Environmental Nanoscience and Risk (CENR). This work was partially supported by National Science Foundation (NSF), and Arnold Doctoral Fellowship.
ABSTRACT

Understanding the environmental factors that affect the fate and effects of engineered nanoparticles (ENPs) in the environment is crucial for NP risk assessment. Silver nanoparticles (Ag NPs) readily transform in the environment, which alters their properties and subsequently their transport, fate, and toxicity. The overall aim of this dissertation was to evaluate the effects of Ag NPs physicochemical properties (e.g., size, surface charge, surface coating) and water chemistry (e.g., buffer concentration, organic ligands, natural organic matter) in controlling Ag NPs colloidal stability. This aim was achieved by systematically 1) review and rationalize many studies that investigated the aggregation kinetics of Ag NPs; and 2) investigate the aggregation kinetics of Ag NPs under specific conditions that has not been studied in the literature, in particular presence of organic ligands with different structures, natural organic matter, and phosphate buffer concentration.

The critical coagulation concentration (CCC) is independent of NP concentration for pure electrostatic interactions. However, in the presence of chemical constituents of high affinity to NPs such as cystine, carbonate, and phosphate anions, the CCC is NP concentration-dependent. Although sterically stabilized Ag NPs do not aggregate even at high ionic strength; they are prone to destabilization following surface coating replacement by molecules with higher affinity to Ag NPs that do not provide steric stabilization. For instance, sterically stabilized polyvinylpyrrolidone (PVP) Ag NPs remained colloidally
stable at high Na\(^+\) and Ca\(^{2+}\) concentrations, but the presence of cystine destabilized PVP Ag NPs at similar Na\(^+\) and Ca\(^{2+}\) concentrations.

The molecular structures of organic ligands play a significant role in determining the stability of Ag NPs. L-cysteine (L-cys) decreases the stability of Ag NPs, whereas N-acetyl-L-cysteine (NAL-cys) increases the stability of Ag NPs. Whereas Suwanee River fulvic acid (SRFA) increased the stability of Ag NPs, addition of a mixture of SRFA and L-cys decreased the stability of Ag NPs. Cystine significantly impacts the stability of citrate, PVP, and polyethylene glycol (PEG) coated Ag NPs, resulting in a concentration-dependent aggregation of Ag NPs, with a shift in the CCC toward lower concentrations of cystine at lower concentrations of Ag NPs. Furthermore, the CCC for Ag NPs decreased with the increase in phosphate buffer concentration in the presence and absence of SRFA.

The majority of NP aggregation studies have focused on the effect of counter ions on NP aggregation kinetics, typically at environmentally irrelevant, high NP concentrations. This PhD focused on the impact of the less studied, yet equally important, environmental factors such as buffers, anions, organic ligands with different structures and properties (e.g., L-cys, NAL-cys and cystine) on Ag NPs stability, especially at environmentally relevant NP concentrations. The findings of this PhD are important to underpin NP risk assessment and environmental fate and behavior studies.
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CHAPTER 1

INTRODUCTION
1.1. Nanotechnology, nanoparticles, and the environment

Engineered nanoparticles (NPs) are defined as purposefully produced particles that have dimensions from 1 to 100 nm, with unique and novel properties (e.g. mechanical, optical, reactivity, etc.) compared to the bulk materials with the same chemical composition.¹ These novel properties have been, and continue to be exploited widely for application in medicine², ³, consumer products⁴, ⁵ (e.g. cosmetics and clothes), and environmental remediation⁶. Consequently, nanotechnology is a rapidly growing industry with increased production volumes of engineered NPs (ENPs)⁷, and thousands of nano-enabled products.⁸ Thus, NPs release from consumer products are inevitable resulting in NPs exposures in the environment. Hence, NPs are listed as emerging contaminants of environmental concerns (CECs)⁹ and there is an imminent need for in-depth understanding of NP environmental and biological interactions. Understanding the environmental fate, behavior and transformation of NPs is a research priority of interest from a fundamental point of view as well as for regulatory agencies and the general public.

1.2. Silver nanoparticles application and release

Silver nanoparticles (Ag NPs) are among the most frequently used ENPs in consumer products due to their antimicrobial, electrical, and optical properties.¹⁰ Ag NPs can be released from nano-enabled products through usage and disposal and find their way into the environment, resulting in environmental and human exposure to Ag NPs.¹¹ Several studies reported silver release from different materials including textiles¹², paints¹³, and plastic food containers.¹⁴ Such releases are expected to result in low concentrations (ca. less than 1 µg L⁻¹) of Ag NPs in surface waters.¹⁵, ¹⁶ Several studies reported the potential
toxicity of Ag NPs to a wide range of environmental organisms such as plants\textsuperscript{17}, algae and fungi\textsuperscript{18}, vertebrates\textsuperscript{19} and invertebrates\textsuperscript{20}, and human cells\textsuperscript{21, 22}.

Many of the studies investigating Ag NPs behavior in the literature used high NP concentrations - typically several mg L\textsuperscript{−1} - than those expected in the environment. This is because of the high detection limit of the analytical techniques (e.g. Dynamic Light Scattering (DLS) and UV-Vis spectroscopy) typically used to investigate NP behavior.\textsuperscript{23, 24} Therefore, there is a need to evaluate the validity of previous results at low environmentally relevant concentrations.

Ag NPs are commonly stabilized with various capping agents such as citrate, polyvinylpyrrolidone (PVP), and polyethylene glycol (PEG).\textsuperscript{25-27} Citrate weakly bonds to the NP surface and stabilizes NPs through electrostatic repulsion, whereas PVP and PEG sterically stabilize Ag NPs. Type of stabilizing mechanism and chemistry of capping agent have a profound effect on fate and transport of Ag NPs in the aquatic environment.\textsuperscript{28, 29} Whereas electrostatically stabilized NPs are prone to aggregation in the presence of counter ions, sterically stabilized NPs remain stable even at high counter ion concentrations.\textsuperscript{30, 31}

1.3. Environmental transformations of silver nanoparticles

Silver NPs are susceptible to transform in the dynamic environmental and biological systems. These transformations - such as sulfidation,\textsuperscript{32} aggregation\textsuperscript{24}, dissolution\textsuperscript{10}, and surface coating modifications by organic ligands\textsuperscript{30} - greatly complicate evaluation of the risk associated with NP release into the environment.\textsuperscript{30, 33} Transformation of NPs can impact their core, surface properties, coatings and subsequently their reactivity in the environment.\textsuperscript{34} Figure 1.1 illustrates the critical NPs transformations affecting NPs
interactions. Metallic Ag NPs may be sulfidized under anoxic condition which decrease their solubility, dissolution rate, and therefore toxicity, potentially limiting their short-term environmental impact. Aggregation and dissolution are key transformation processes that determine NP reactivity, bioavailability, toxicity, and environmental fate and effects. Similarly, the interaction between NPs and natural organic matter (NOM) results in a nanoscale coating of the NPs altering their aggregation, deposition, and toxicity. Ag NP transformations depend on NP physicochemical properties (e.g. size, shape, surface charge, and concentration) and the characteristics of the media (e.g. pH, ionic strength, and presence of natural organic macromolecules).

1.3.1. Silver nanoparticle aggregation and sedimentation

According to the DLVO theory, the overall interaction energy between two identical particles is the sum of the van der Waals attraction and the electrical double layer repulsion forces (Figure 1.2). Based on the DLVO theory, when the electrostatic repulsion forces prevails, NPs repel each other and the potential barrier in the primary minimum prevents the NPs aggregation. If the van der Waals attraction dominates, NPs can overcome the potential barrier and aggregation occurs in the primary minimum.

Experimentally, the colloidal stability of any dispersion can be quantitatively determined by measuring the growth of aggregate size overtime; that is the aggregation rate. The attachment efficiency (α) - the inverse of the stability ratio (W) – is typically determined as the rate of slow aggregation to that of the fast aggregation. The minimum counter ion concentration required to fully destabilize the dispersion is defined as the critical coagulation concentration (CCC). The CCC is typically used to compare the overall stability of a suspension and to compare the efficiency of counter ions to destabilize
NP suspensions. In the aquatic environment, Ag NPs colloidal stability is determined by the concentration and valency of counter ions, and NP interaction with NOM. In general, Ag NP tend to remain colloidally stable at low ionic strength, in particular at low multivalent (e.g., Ca$^{2+}$, Mg$^{2+}$) ion concentration. Multivalent counter ions are more efficient in destabilizing NP suspension compared to monovalent counter-ions according to Schulze-Hardy rule. In high ionic strength estuarine and marine waters, NPs behavior is determined by rapid aggregation and sedimentation due to the high ionic strengths. However, individual NPs or small aggregates can persist in the environment for up to several days at high ionic strength such as in sea water most likely due to the stabilization by NOM.

Several NP aggregation kinetics’ experimental studies have focused on investigating the effect of ionic strength, ion valency, pH and NOM on the stability of NPs. However, wide discrepancies can be found among the reported CCC and $\alpha$ values. These inconsistencies are likely to be attributed to the differences in the experimental conditions including: 1) methods used to study NP aggregation kinetics, 2) physicochemical properties of the NPs, and 3) media chemistry.

1.3.1.1.Methods to study silver nanoparticle aggregation

The most commonly used techniques to measure NPs aggregation kinetics are DLS and UV-Vis spectroscopy. For DLS, NP aggregation is measured by monitoring the increase in aggregate hydrodynamic diameter ($z$-$d_h$) of the Ag NPs as a function of time. The aggregation rate constant (k) is proportional to the slope of the hydrodynamic diameter growth. For UV-Vis, NP aggregation is measured by monitoring the changes in the absorption spectrum of Ag NPs as a function of time. Aggregation rate constant is
determined by measuring the rate of the loss of the surface plasmon resonance of primary Ag NPs in aqueous solution.\textsuperscript{39}

1.3.1.2. Effect of NP properties on silver nanoparticle aggregation

There is currently a limited, and often contradictory understanding of the effect of Ag NPs intrinsic properties (and other NPs in general) and medium, other than ionic strength, ion valency and NOM on the stability of Ag NPs. For instance, recent studies reported contradictory results on the dependence of CCC on NP size including a decrease with the decrease in NP size (e.g. hematite\textsuperscript{49}, TiO\textsubscript{2}\textsuperscript{50}), an increase with the decrease in NP size (e.g. CdSe\textsuperscript{51}) or independence of CCC of NP size (e.g. Au NPs\textsuperscript{52}). However, no studies investigated the effect of Ag NPs size on their stability.\textsuperscript{38}

The capping agent is the factor that determines NP stabilization mechanism.\textsuperscript{48} El Badawy et al. (2010) showed that the type of stabilizing mechanism profoundly impacts the aggregation potential of Ag NPs.\textsuperscript{29} Physicochemical properties of the capping agent have a greater influence on the aggregation behavior of functionalized Ag NPs than either core composition or their size.\textsuperscript{38} Some capping agents such as citrate stabilize NPs by electrostatic mechanism, whereas other capping agents such as PVP stabilize NPs by steric mechanisms. Generally, the CCC of charge-stabilized Ag NPs are lower than those of sterically-stabilized Ag NPs, and some sterically stabilized Ag NPs are kinetically stable at even very high electrolyte concentrations.

1.3.1.3. Effect of media Chemistry on silver nanoparticle aggregation

The physicochemical properties of water – such as ionic strength, counter-ion concentration and valency, co-ions, NOM concentration and composition and pH – are key
determinant of NP aggregation behavior.\textsuperscript{28, 53} The increase in ionic strength of the media results in an increases in the aggregation of uncoated and electrostatically-stabilized NPs as previously reported.\textsuperscript{29} Also multivalent counter-ions are more efficient in destabilizing NP suspension compared to monovalent counter-ions according to Schulze-Hardy rule.\textsuperscript{44} Surface charge titration and electrostatic double layer (EDL) screening are the two primary ways that pH and ionic solutes promote NP aggregation. High ionic concentration decreases the Debye length ($\kappa^{-1}$), resulting in the decrease of the extent of EDL repulsion. The ionic concentration where the repulsive energy barrier is completely screened and rapid aggregation occurs is known as the CCC.\textsuperscript{46} However the ionic strength and the electrolyte type had no effect on the aggregation of sterically stabilized Ag NPs.\textsuperscript{29}

NOM sorbs on the NP surface resulting in the formation of a surface coating which typically enhances NPs colloidal stability due to the electrosteric stabilization, however NP destabilization in the presence of NOM due to the bridging mechanism at high divalent counter-ion concentrations has been observed.\textsuperscript{46} Also, Some organic ligands such as L-cysteine has been shown to alter the NP aggregation behavior.\textsuperscript{54} The surface charge and aggregation of Ag NPs have been shown to be affected by the solution pH\textsuperscript{29}; however, little is known about the effect of buffer on NP aggregation in the literature despite being widely used to control the pH of the dispersion.

Buffers are commonly used to maintain a constant pH during a reaction, but their presence can result in substantial changes in NP surface chemistry\textsuperscript{55} and stability\textsuperscript{56}. Few studies investigated the impact of buffer concentration on NP aggregation. For instance, increasing 3-(N-Morpholino)propanesulfonic acid (MOPS) buffer concentration resulted in an increased goethite NPs aggregate size, which was attributed to sorption of MOPS
molecules on the surface of goethite NPs accompanied by a reduction in surface charge.\textsuperscript{56} Furthermore, the buffer can greatly affect the aggregation state of goethite NPs. Goethite NPs suspended in solution containing 1 mM Fe\textsuperscript{2+} and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer were the most dispersed, and the aggregate sizes were 3–5 times larger for goethite NPs suspended in MOPS and Triethanolamine (TEA) buffers.\textsuperscript{56} The stability of hematite particles decreases in the presence of phosphate anions.\textsuperscript{57} Therefore, while buffers enable adequate maintenance of pH, their presence is not passive with regard to NP surface and aggregation kinetics. However, the impact of buffer type and concentration on the aggregation kinetics of NPs has not been investigated.

1.3.1.4 Effect of NOM on silver nanoparticle aggregation

NOM sorbs on the NP surface resulting in the formation of a surface coating that can substantially alter NP aggregation behavior. The NP behavior and effects depends on the composition and concentrations of the NOM.\textsuperscript{58} NOM is ubiquitous in natural environments and varies in concentration from 0.1 to 10s of mg L\textsuperscript{-1}, depending on biogeochemical and climatic conditions.\textsuperscript{59–61} NOM is composed of a complex mixture of polyelectrolytic and polyfunctional organic molecules that vary both spatially and temporally in terms of molecular composition, acidity, molecular weight, structure, and charge density.\textsuperscript{62} The main components of NOM are humic substances, polysaccharides, lipids, proteins and other classes of organic molecules.\textsuperscript{60,61} The widely diverse composition and properties of NOM (e.g. N- and S-content, functional groups, molecular weight, hydrophobicity, etc.) will significantly impact the molecular composition of NP surface coating, thereby influencing NP fate, behavior, bioavailability and toxicity.\textsuperscript{63–65}
1.4. Dissertation overview

In short, the fate and behavior of Ag NPs in environmental and biological media remain uncertain. In particular, the impact of NP physicochemical properties (e.g., particle size, capping agent, etc.) and environmental conditions (pH, ionic strength, availability of free sulfide, etc.) on NP aggregation kinetics is not fully understood yet. Therefore, the overall aim of this thesis is to investigate the effects of NPs and media physiochemical properties on Ag NPs aggregation kinetics.

Chapter 2 provides a literature review of the various parameters affecting Ag NP aggregation behavior in aqueous media. It provides quantitative description of the effect of NP size and buffer concentration on NP aggregation kinetics and qualitative description of other NP properties and media physicochemical properties. Finally, this review highlights the key data and knowledge gaps in understanding Ag NP aggregation kinetics.

Chapter 3 investigates how cystine affects the stability of citrate-, PVP-, and PEG-coated Ag NPs with and without Suwannee River fulvic acid (SRFA) as a function of Ag NPs concentration. The results demonstrated, for the first time, the concentration-dependent aggregation of citrate-coated Ag NPs (cit-Ag NPs) in the presence of cystine with a shift in the CCC to lower cystine concentrations at lower cit-Ag NPs concentrations.

Chapter 4 describes the aggregation and dissolution behavior of cit-Ag NPs in the presence of L-cysteine (L-cys) and N-acetyl-L-cysteine (NAL-cys). It also investigates the effect of SRFA and a NOM isolated from the Yukon River (YRNOM) on the stability of cit-Ag NPs. Results demonstrated that the molecular structure of the organic ligands play a significant role in determining the stability of NPs. L-cys decreases the stability of Ag NPs, whereas NAL-cys increases the stability of Ag NPs. SRFA and YRNOM enhanced
the stability of cit-Ag NPs and increased the CCC value to higher counter-ion concentrations.

Chapter 5 investigates the effect of phosphate buffer on aggregation kinetics of cit-Ag NPs induced by Na\(^+\) and Ca\(^{2+}\) as counter-ions in the presence and absence of SRFA. Results demonstrate that phosphate buffer, which serves to maintain a constant pH, is an important determinant of NP aggregation behavior.

Chapter 6 discusses the overall conclusions of the dissertation, the environmental implications of the study and the future recommendations.
Figure 1.1. Transformations of Ag NPs in the environment.\textsuperscript{30}
Figure 1.2. Total interaction energy between NPs in a liquid medium according to DLVO theory (https://creativecommons.org/licenses/by/3.0/).
CHAPTER 2

EFFECT OF NANOMATERIAL AND MEDIA PHYSICOCHEMICAL PROPERTIES ON AG NM AGGREGATION KINETICS

Abstract

Nanomaterial (NM) aggregation is a key process determining their environmental, fate behavior and effects. Nanomaterials are typically engineered to remain kinetically stable; however, in environmental and toxicological media, NMs are prone to aggregation. The aggregation kinetics of NM is typically quantified by measuring their attachment efficiency ($\alpha$) and critical coagulation concentration (CCC). Several studies measured $\alpha$ and CCC for Ag NMs with a major focus on investigating the effects of ionic strength, ion valency and natural organic matter, with few studies investigating other environmental factors such as light and dissolved oxygen and none investigating the effect of particle size, buffer type and concentration, or surface coverage by capping agent. A survey of recent research articles reporting CCC values for Ag NMs reveals substantial variation in experimental conditions and particle stability with CCC values of monovalent and divalent counterions covering a wide range (ca. 25 to infinity for monovalent counterions and 1.6 to infinity for divalent counterions).

Here, we rationalize the differences in the CCC values for Ag NMs based on the variability in the experimental conditions, which includes NM and medium physicochemical properties. Capping agents determine NM stability mechanism with citrate, sodium dodecyl sulfate (SDS), and alginate stabilizing NM by electrostatic
mechanism; whereas polyvinylpyrrolidone (PVP), casein, dextrin, tween, branched polyethyleneimine (BPEI), and Gum Arabic stabilizing NMs by steric mechanisms. The CCC values for Ag NMs with different capping agents follow the order citrate ~ alginate ~ SDS < casein < dextrin < PVP < tween < branched polyethyleneimine (BPEI) ~ gum Arabic. For charge stabilized Ag NMs, the CCC increases with the decrease in NM size and buffer concentration and decreases with light irradiation. For sterically stabilized PVP-Ag NMs, the CCC increases with the coating concentration/surface coverage and completely coated Ag NMs do not undergo aggregation even at high salt concentrations.

2.1. Introduction

Understanding NM stability, fate and behavior in environmental and toxicological media is crucial to allow for comprehensive environmental risk assessment of NMs. Aggregation is one of the key processes, among others, determining their environmental fate, behavior, uptake, and effects. Furthermore, understanding the interplay of NM- and medium- physicochemical properties and their aggregation behavior under conditions of ecotoxicological and environmental media is required for an adequate interpretation of ecotoxicological test outcomes, fate and behavior studies, to generate parameters (e.g. attachment efficiency and critical coagulation concentration, $\alpha$ and CCC, respectively) for environmental fate models, and for comparison among different studies.

One of the quantitative methods to assess the stability of any dispersion is to measure the attachment efficiency ($\alpha$) - the inverse of the stability ratio ($W$) - and the CCC, which is the minimum electrolyte concentration required to fully destabilize the dispersion. The $\alpha$ and CCC parameters of a specific counterion is commonly described by the classical DLVO theory on the basis of a static force balance. The DLVO theory
attributes the interaction between two identical particles to van der Waals attraction and the electrical double layer repulsion\textsuperscript{8,9}. A substantial progress in the understanding of the stability phenomena has been made since the introduction of the DLVO theory. Additionally, numerous studies have shown the importance of other interaction forces such as steric forces, hydration, etc\textsuperscript{10}. The CCC can, however, also be estimated from a kinetic point of view by studying the process of colloidal coagulation, or from a dynamic point of view by considering colloidal transport in non-equilibrium systems where other processes such as diffusion and the influence of gravity come into play\textsuperscript{11}. Several Ag NM aggregation kinetics’ experimental studies have focused on investigating the effect of ionic strength, ion valency, pH and natural organic matter (NOM) concentration on the stability of Ag NMs (CCC and $\alpha$)\textsuperscript{12-16}. However, wide discrepancies can be found in the literature among the reported CCC and $\alpha$ values (Table 2.1). The CCC values of monovalent and divalent counterions for Ag NMs found in the literature cover a wide range (ca. 25 to $\alpha$ for monovalent counterions and 1.6 to $\alpha$ for divalent counterions). These differences are likely to be attributed to the differences in the experimental conditions among the different studies including: 1) method used to study NM aggregation kinetics (dynamic light scattering, DLS, and UV-Vis spectroscopy)\textsuperscript{16}, 2) physicochemical properties of the NMs (e.g. size, shape, surface coating, etc.)\textsuperscript{17,18}, and 3) media chemistry (buffer type and concentration)\textsuperscript{19}.

There is currently a limited, and often contradictory, knowledge on the effect of intrinsic properties of Ag NMs (and other NMs in general) and medium, other than ionic strength, ion valency and NOM on the stability of Ag NMs. For instance, recent studies reported contradictory results on the dependence of CCC on NM size including a decrease with the decrease in NM size (e.g. hematite\textsuperscript{20}, TiO$_2$\textsuperscript{18}), an increase with the decrease in
NM size (e.g. CdSe\textsuperscript{21}) or independence of CCC of NM size (e.g. Au NMs\textsuperscript{22}). However, no studies in the literature investigated the effect of Ag NMs size on their stability. In addition, whereas some studies reported a linear correlation between the CCC and NM primary particle size (e.g. anatase TiO\textsubscript{2}\textsuperscript{18}), other studies found that the CCC correlated better with NM specific surface area (e.g. anatase TiO\textsubscript{2}\textsuperscript{18}, CdSe\textsuperscript{21}). Other studies reported an important role of stabilizing agent\textsuperscript{21}, impurities introduced during synthesis process\textsuperscript{23}, etc. on NM stability. These studies suggest that material properties such as particle size, capping ligand, impurities, and other properties are important parameters affecting NM stability. However, a systematic review/analysis of the role of intrinsic properties of Ag NM on their aggregation kinetics is lacking.

In this context, this article aims to 1) investigate the relationship between the CCC values for Ag NMs and the parameters affecting Ag NM aggregation behavior and kinetics, and 2) rationalize the discrepancies in the reported CCC values for Ag NMs in the literature. This article also provides a critical discussion on deviations from DLVO theory due to NM specific properties.

2.2. Methods

2.2.1. Data collection

Data on Ag NM properties (size, zeta potential, coating type and concentration, and NM concentration), water physicochemical properties (pH, buffer concentration, ion type and concentration, concentration and type of NOM) and CCC were extracted from research articles reporting Ag NM aggregation kinetics’ (11 articles) experiments and deriving CCC values (55 measurements) and tabulated (Table 2.1 and 2.2). Ag NMs were selected for
this review as they are the most widely studied NMs in the literature with the largest number of articles reporting CCC values under variable NM and media properties.

2.2.2. Data analysis

The collected data for Ag NMs were separated into electrostatically and sterically stabilized NMs to facilitate data discussion, interpretation and rationalization of differences. The relationships between NM physicochemical properties, media physicochemical properties and CCC were examined for both electrostatically and sterically stabilized NMs by regression analysis. Due to the relatively small sample size (range 4-18) and the non-linear association (e.g. Figure 2.1 and Figure 2.4), the Spearman’s non-parametric rank correlation test was used to test if there exist significant relationships between NM physicochemical properties/media physicochemical properties and CCC under various experimental conditions. The statistical significance was set as p-value < 0.05.

2.2.3. Zeta potential measurement

Citrate-coated Ag NMs were synthesized and characterized using a previously reported method\textsuperscript{24}. The properties of these citrate-coated Ag NMs are reported elsewhere\textsuperscript{25}. Briefly, the size and electrophoretic mobility (EPM) of Ag NMs were measured by dynamic light scattering (DLS) and laser Doppler electrophoresis, respectively using a Malvern Zetasizer NanoZS Instrument (Malvern, USA). According to DLS, the z-average hydrodynamic diameter (\(z-d_h\)) and polydispersity index of synthesized citrate-coated Ag NMs in stock suspension were 24.48 ± 0.10 nm and 0.21 ± 0.05, respectively. The zeta
potential of the synthesized Ag NMs was -41.7 ± 2.0 mV at approximately pH 7.0 using Smoluchowsky approximation. The standard deviation reported here shows the stability of the replicate measurements and was determined from 5 replicates of size and 10 replicates of zeta potential.

Polyvinylpyrrolidone coated Ag NMs (PVP-Ag NMs) with an increased number of PVP molecules per Ag NM unit surface area were prepared to evaluate the effect of PVP surface coverage on the zeta potential of PVP-Ag NMs. Briefly, PVP-Ag NMs were prepared by ligand exchange approach using citrate-coated Ag NMs as precursor. Aliquots of Citrate-coated Ag NMs (Cit-Ag NMs) were converted into PVP-Ag NMs by adding 4-16 molecules of PVP per nm$^2$ surface area of cit-Ag NMs under vigorous stirring (e.g. 700 rpm) for at least 1 hour and then cooled overnight at 4°C. The electrophoretic mobility of Ag NMs was measured by laser Doppler electrophoresis using a Malvern Zetasizer NanoZS Instrument (Malvern, USA). The pH of all Ag NM suspension was routinely measured and was approximately 7.0 ± 0.2.

2.2.4. DLVO calculations

Classical DLVO calculations were performed to underpin the observed results according to the equations presented in$^{15,26}$ and according to the following assumptions: (i) ionic strength of $10^{-3}$ M monovalent electrolyte, (ii) the zeta potential is constant at -42 mV, (iii) steric stabilization induced by capping agents is not considered.

2.3. Results and discussion

A survey of recent research articles in the field reveals substantial variation in experimental conditions (Table 2.1). Variations include NM size, concentration, surface
coating type and concentration, buffer concentration, electrolyte type and concentration, dissolved oxygen (DO), and light. For instance, Z-average hydrodynamic diameter ranged from 21 to 107.5 nm, number average NM size ranged from 5.4 to 82.0 nm, NM concentration ranged from 0.4 to 24.5 mg L\(^{-1}\), buffer concentration ranged from 0 to 0.15 mM NaHCO\(_3\), and capping agents includes citrate, alginate, SDS, casein, dextrin, PVP, tween, BPEI, and gum Arabic. Small variations in reaction conditions may be largely irrelevant or may fundamentally change the fate and behavior of NMs such that comparisons between datasets become challenging. The CCC values of monovalent and divalent counterions for Ag NMs found in the literature cover a wide range (ca. 25 to infinity for monovalent counterions and 1.6 to infinity for divalent counterions; Table 2.1). Below we rationalize the differences in the CCC values based on the variability in the experimental conditions, which includes NM and medium physicochemical properties.

2.3.1. Capping agent

Overall, the CCC values of Ag NMs with different capping agents follow the trend citrate ~ alginate ~ SDS < casein < dextrin < PVP < tween < BPEI < gum Arabic (Table 2.1). The capping agent is the factor that determines NM stabilization mechanism\(^{2,27}\). Physicochemical properties of the capping agent have a greater influence on the aggregation behavior of functionalized Ag NMs than either core composition or their size (Table 2.1)\(^{22}\). Some capping agents such as citrate, SDS, and alginate stabilize NM by electrostatic mechanism\(^{16,28-32}\); whereas other capping agents such as PVP, casein, dextrin, tween, branched polyethyleneimine (BPEI), and Gum Arabic stabilize NMs by steric mechanisms\(^{29,32,33}\). Generally, the CCC of charge stabilized Ag NMs are lower than those of stERICALLY-stabilized Ag NMs, and some sterically stabilized Ag NMs are kinetically
stable at even very high salt concentrations (ca. up to 10 M NaNO₃ and 8 M CaCl₂). The dependence of the CCC values on Ag NM and media properties are discussed for the two categories based on the stability mechanisms of Ag NMs: charge- and sterically-stabilized NMs.

2.3.2. Behavior of Charge-Stabilized NMs

For charge-stabilized Ag NMs (e.g. bared, citrate, SDS, and alginate coated), the CCC values show a wide discrepancy ranging from 30 to 122 mM NaCl, 25 to 200 mM NaNO₃, 1.6 to 2.2 mM CaCl₂ and 1.7 to 5 mM Ca(NO₃)₂. The differences in the CCC values are likely due to the differences in NM properties (e.g. NM size, concentration, capping agent concentration) and medium chemistry (e.g. counter-ion types, buffer concentration, etc.). Charge-stabilized NMs owe their stability to particle charge, which results in the formation of diffuse double layer around NMs. Any constituent which influences the surface charge will also change NM stability. The most profound effect is exercised by ions of charge opposite to that of NMs, which result in charge screening and shrinkage of the diffuse double layer thickness. Yet counterion charge is not the only factor influencing the stability of a given NM. Size, shape, degree of hydration, character of the ligands in complexes and other parameters play important roles. However, little attention has been given to the effects of these factors on NM aggregation kinetics in the literature.

2.3.2.1. Counterion and natural organic matter

The effects of counterions, pH and NOM on Ag NM aggregation kinetics are the most widely studied and understood (Table 2.1), and therefore are only briefly summarized here. Divalent counterions (cations or anions) are more efficient in screening NM surface
charge than monovalent counterions, in good agreement with Schulze-Hardy rule\textsuperscript{35-37}. For a typical electrostatically stabilized NM, the CCC of an electrolyte depends strictly on the counterion charge, as long as the adsorption of the co-ion or the counterions on the surface is negligible. This is the condition when the Schulze-Hardy rule is obeyed. If an ion adsorbs sufficiently strongly at concentrations lower than the CCC, the Schulze-Hardy rule does not apply\textsuperscript{34}. The majority of studies on electrostatically stabilized Ag NMs reported CCC values for monovalent and divalent electrolytes (\textit{ca.} $\frac{\text{CCC}_{\text{mono}}}{\text{CCC}_{\text{di}}} = 8-43$\textsuperscript{30,38-41}) within the boundaries (\textit{ca.} $\frac{\text{CCC}_{\text{mono}}}{\text{CCC}_{\text{di}}} = 4-64$) of the Schulze-Hardy rule\textsuperscript{35,37}.

Natural organic matter (typically Suwannee River fulvic and humic acids) increases the CCC of Ag NMs by 1.4-4.3 folds (Table 2.1 and 2.2). This is because NOM replaces the capping agent (typically citrate) and impact electrosteric stabilization of Ag NMs\textsuperscript{42}. Additionally, several studies have demonstrated that NOM results in a reduction in the Hamaker constant\textsuperscript{16,30,43}.

2.3.2.2. Size

The effect of size on CCC and $\alpha$ of Ag NMs has not been investigated explicitly in any study in the literature. Taking all CCC values reported in the literature for charge stabilized Ag NMs, clear relationship between NM size and CCC can be determined. For monovalent electrolytes, the CCC shows a strong association between NM hydrodynamic diameter measured by DLS (P value = 0.0075 and 0.00499 for NaCl and NaNO\textsubscript{3} counterions; Figure 2.1a), or primary NM size measured by microscopy techniques (P value = 0.0735 for NaNO\textsubscript{3} counterions; Figure 2.1c). The CCC increase with the decrease in NM size, with a sharp increase in the CCC at approximately a z-average hydrodynamic
diameter of 50 nm and a core size of approximately 20 nm. This behavior is in agreement with a previous study on citrate-Au NMs, where Frens observed a marked increase in the CCC (from 5 to 70 mM NaNO₃) of citrate-Au NMs with the decrease in particle size (from 70 to 7.5 nm). They used this phenomenon to fractionate a mixture of particles (7.5 and 80 nm) according to particle size by coagulating the larger particles by addition of 30 mM NaNO₃. Despite the strongly negative association between the CCC and particle size for monovalent electrolytes (Figure 2.1a and c, Table 2.3), no clear trend was observed between CCC and Ag NM size for divalent electrolytes (Figure 2.1b and d, Table 2.3). The Spearman’s correlation tests were presented in Table 2.3.

The increase in the CCC with the decrease in Ag NM size seems to be in contradiction with the general notion that the interaction energies of NM during slow coagulation should be proportional with the particle size and that, therefore, larger particles are expected to be more stable under equal experimental conditions. However, this notion applies only for slow coagulation regime (reaction limited aggregation regime; Figure 2.2) and does not affect the CCC value. Figure 2.2 illustrates that according to DLVO theory, under RLA regime, the attachment efficiency decreases with the increase in NM size, but the CCC remains constant. This argument has been misleadingly used in several publications to justify the increase in CCC with the increase in NM size.

Recent studies reported contradictory results on the dependence of CCC on NM size including a decrease with the decrease in NM size (e.g. hematite, TiO₂), an increase with the decrease in NM size (e.g. CdSe) or independence of CCC of NM size (e.g. Au NMs). Similarly, earlier studies on colloid stability reported contradictory results including a decrease, an increase, an increase followed by a decrease, as well as
an independence of colloid stability on particle size. These contradictory results were attributed, at least partly, to deviations from sphericity and contributions of surface irregularities rather than to differences in particle size. Other explanations such as distribution of surface potential, hydrodynamic interaction, dynamics of interaction, deposition in secondary minimum, and surface roughness have been discussed in details elsewhere.

For instance, some studies accounted for the decrease in CCC with the increase in particle size by considering secondary minimum flocculation in the available models, where small particles aggregate in primary minimum, whereas large particles aggregate in secondary minimum. Secondary minimum in the total energy of interaction profile can occur at moderate to large separations (usually larger than several nanometers). For a given medium properties, the depth of the secondary minimum increases with particle size (Figure 2.3). Coagulation of Brownian particles in secondary minimum is possible, in principle, if the resultant of the force from the thermal energy of the particles and the fluid drag is insufficient to drive the particles out of the secondary minimum. Indirect evidence for this phenomenon has been reported by numerous investigators. For instance, the CCC of hematite particles (ca. 74 to 704 nm) as a function of particle size shows a minimum at a diameter of about 400 nm. The small particles probably coagulate in the primary minimum, whereas for the large particles secondary minimum flocculation is dominant. The increase in CCC for d > 400 nm was explained by shear induced aggregate disruption for electrolyte concentrations below the CCC.

Another reason for such a deviation from classical-DLVO prediction of size-independent CCC values is the assumptions used in DLVO theory. The derivation of the
DLVO theory was based on the assumptions that the thickness of the electrical double layer ($\kappa$) surrounding a charged particle in an electrolyte solution is much smaller ($r$) than the linear size of the particle ($\kappa r \gg 1$), and the electrical interaction energy can be estimated through the superposition principle. The former implies that the CCC is independent of the size of a particle, and the latter assumes that two interacting particles can be considered independently. Hsu and Liu, examined the assumptions made by the DLVO theory by investigating the electrical interaction between two spherical particles as a function of particle size at constant surface potential and considering the case that the thickness of the electrical double layer is not necessarily much smaller than the radius of a particle. They concluded that, at constant surface potential, if the radius of a particle is $> 1 \mu$m, CCC is independent of particle size; whereas for smaller particles, the CCC increases with the decrease in particle size. This deviation was attributed to the application of the superposition principle in the evaluation of the electrical interaction energy.

Furthermore, the contradictory results in the literature regarding the effect of NM size on the CCC values can be rationalized by taking into account the variability in NM surface charge (e.g. zeta potential). It is clear that in the studies reporting a decrease in CCC with the increase in NM size, the NMs are characterized by a narrow range of surface potential (ca. zeta potential of -33 ± 3 to -35 ± 5 for CdSe NMs); whereas the studies reported an increase in CCC with the increase in NM size, or a size-independence of CCC, NMs are characterized by variable zeta potential.

For instance, the similarity in the CCC values of 30 and 100 nm Au NMs reported by Liu et al can be attributed to the differences in the zeta potential of the pristine particles (ca. -42 and -50 for the 30 and 100 nm NMs, respectively), which might compensate for
the differences in NM size. That is, the increased zeta potential of the 100 nm could compensate for the expected reduction in CCC value, thus CCC value for the 30 and 100 is the same. Similarly, the surface charge of TiO$_2$ NMs decrease (become more negative) with the increase in NM size, thus the increase in the CCC value with the increase in NM size. The decrease in hematite NM CCC (70±6, 54±9, and 45±4) with the decrease in NM size (65, 32 and 12 nm) can be attributed to the decrease of zeta potential of NMs (ca. 33±2.7, 24±2.9, and 15.1±1.7, respectively) as particle size decreases. Therefore, in these studies, the correlation between NM size and CCC could be influenced by the variability of the surface charge, and cannot be attributed solely to NM size.

It is noteworthy the zeta potential of all charge-stabilized Ag NMs (Table 2.1) varies within a tight range (ca. -35 to -48), except two NMs (-22 to -26.5), which can explain the differences in the trend of CCC with size for charge stabilized Ag NMs compared to other studies. Additionally, larger number of NMs is considered in this review allowing accounting for the variability in the experimental conditions.

2.3.2.3. Buffer concentration

Buffers are commonly used to maintain a constant pH during a reaction, but their presence can result in substantial changes in surface chemistry and NM stability. Few studies investigated the impact of buffer concentration on NM aggregation. For instance, increasing 3-Morpholinopropanesulfonic acid (MOPS) buffer concentration resulted in an increased goethite NMs aggregate size, which was attributed to sorption of MOPS molecules on the surface of goethite NMs accompanied by a reduction in surface charge. Furthermore, the buffer identity greatly affects the aggregation state of goethite NMs.
Goethite NMs suspended in solution containing 1 mM Fe$^{2+}$ and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer were the most dispersed, and the aggregate sizes were 3-5 times larger for goethite NMs suspended in MOPS and Triethanolamine (TEA) buffers$^{19}$. The stability of hematite particles decrease in the presence of phosphate anions$^{59}$. Therefore, while buffers enable adequate maintenance of pH, their presence is not passive with regard to NM surface and aggregation kinetics. However, there is no single study that investigated the impact of buffer type and concentration on the aggregation kinetics of NMs in the literature.

Buffered (carbonate buffer) Ag NMs have lower CCC values compared to unbuffered Ag NMs and the CCC values decrease with the increase in buffer concentration (Table 2.1 and Figure 2.4a). The Spearman’s correlation test (Table 2.3) showed a significant negative correlation between buffered Ag NMs and the CCC values (P value = 0.0070 for NaCl). For NaNO$_3$, the Spearman’s correlation test (Table 2.3.) did not show a significant negative correlation between buffered Ag NMs and the CCC values (P value = 0.8089), which is likely due to the small number of samples and the effect of NM size on the CCC in addition to the effect of buffer. These observations suggest that pH buffers are likely to play an important role in controlling NM stability and need to be further investigated. This is consistent with the high affinity of carbonate anions to silver ($K_{sp}$ at 25 °C= $8.46 \times 10^{-12}$ M$^2$), which may result in the formation of Ag$_2$CO$_3$ layer on the surface of Ag NMs, altering NM surface charge and Hamaker constant and thus Ag NM stability. At pH 7, the zeta potential of Ag NMs increases (become less negative) with the decrease in NM concentration (1 to 100 µM) in 10 mM carbonate concentration$^{60}$. Concurrently, Ag NM aggregate size increases with the decrease in NM concentration. This behavior can be
attributed to the increased sorption of \( \text{CO}_3^- \) anions on the surface of Ag NMs at lower NM concentrations due to the increased abundance of \( \text{CO}_3^- \) anions compared to NM total surface area.

2.3.2.4. Chemical interactions of NMs with water constituents

Some medium constituents have very high chemical affinity to a wide spectrum of NMs. Taking silver as an example; \( \text{Ag}_2\text{S} \) have a stability constant of \( 8 \times 10^{-51} \text{ M}^2 \), \( \text{Ag}_3\text{PO}_4 \) of \( 8.9 \times 10^{-17} \text{ M}^2 \), \( \text{Ag}_2\text{CO}_3 \) of \( 8.46 \times 10^{-12} \text{ M}^2 \), etc. These strong interactions of NMs with these constituents are likely to form a surface layer of Ag complexes, resulting in the formation core-shell NMs, and thus controls NM surface charge and aggregation in these media and some emerging studies have demonstrated these effects\(^6\).

The chemical sorption of macromolecules, anions, buffers, \textit{etc}. has been widely ignored in studying NM aggregation kinetics. However, some studies have reported the effect of some of these parameters on NM/aggregate size\(^{61-68} \). For instance, addition of cystine to citrate-stabilized Ag NMs results in NM concentration-dependent shift in the CCC, where the CCC as mM cystine decreases with the decrease in Ag NM concentration\(^{31} \). Organic compounds containing both thiol and amine groups strongly promote the aggregation of citrate-coated Au NMs due to their cooperative functionalities\(^{66} \). Three features of organic compounds could influence citrate-stabilized Au NMs (and similarly Ag NMs); the presence of a sulfur atom, which can form covalent bonds with gold atoms, the presence of ionizable functional groups, and the sign (+ or -) of ionizable functional groups\(^{66} \).

Furthermore complexation of the counterion in electrolyte mixtures may result in an increase in the measured CCC values. For instance, Matijevic and Stryker (1966)
reported an increase in CCC of Aluminum ion for negatively charged silver iodide sols if the sol is acidified by sulfuric acid; whereas this behavior was not observed when nitric acid or perchloric acids are used to lower the pH\textsuperscript{69}. This behavior was attributed to the formation of AlSO\textsubscript{4}\textsuperscript{+} complexes under acidic conditions resulting in the reduction in the concentration of Al\textsuperscript{3+} ions\textsuperscript{70}, according to Schulze-Hardy rule monovalent ions are orders of magnitude less efficient in causing particle aggregation compared with trivalent ions. These two studies reveal the importance of considering counterion speciation in interpreting the variation in CCC.

2.3.2.5. Dissolved oxygen

Dissolved oxygen (DO) in the aqueous environments tend to oxidize metallic NMs such as Ag NMs and QDs\textsuperscript{71-74}. The oxidation invokes several complex physicochemical processes that may occur simultaneously such as formation of a surface oxide layer (\textit{e.g.} AgO\textsuperscript{75}), release of the metallic cations\textsuperscript{72,75,76}, complexation and speciation of metallic cations with aqueous components such as NOM, and aggregation\textsuperscript{71,72,77}. For instance, the aggregation rate of Ag NMs is much faster (\textit{e.g.}, 3–8 times depending on the primary particle size) in the presence of DO than those without DO. The hydrodynamic diameter of Ag NMs had a linear growth within the initial 4–6 h and after the linear growth, the hydrodynamic sizes became random for Ag NMs in the presence of DO, whereas in the absence of DO the hydrodynamic sizes grew smoothly and steadily\textsuperscript{71}. The hydrodynamic diameter of Ag NMs increased more drastically and randomly in the presence of DO, probably due to Ag\textsuperscript{+} ion release and particle debris formation; whereas in the absence of DO, the hydrodynamic diameter increased relatively slower and linearly. The increased
aggregation of Ag NMs in the presence of DO was attributed to an unknown mechanism such as surface energy changes due to adsorption of Ag$^+$ or surface oxidation$^{71}$. Surface oxidation of Ag NMs has been reported to increase the zeta potential of Ag NMs (become less negative)$^{78}$, which could be due to the local increase in Ag$^+$ at the surface of Ag NMs during the dissolution process, resulting in surface charge neutralization and Ag NM aggregation. Furthermore, NM aggregation can be related to the degradation/detachment of surface coating in the presence of oxygen. For instance, the destabilization of Ag NMs, CdS NMs and QDs was found to be related to the detachment/degradation of surface coating in the presence of oxygen, compression of EDL and surface energy changes$^{72,77,79}$. In contrast, anoxic and anaerobic conditions exert low redox potentials, which inhibit oxidation and consequently lead to different aggregation kinetics$^{72,77}$.

2.3.2.6. Light

Light exposure is another important environmental factor affecting NM aggregation behavior and other NM physicochemical processes$^{72,80-82}$. Light irradiation such as UV, Xenon lamp, solar and even fluorescent light has been shown to promote NM surface oxidation, dissolution and aggregation$^{80,83,84}$. For instance, the CCC of NaNO$_3$ for citrate-Ag NMs decreased with 3-day exposure to UV radiation compared with unexposed Ag NMs. The CCC values for fresh, 3 day UVA (320-400 nm)- and 3 day UVB (280-320 nm)-aged Ag NMs were 161.4, 37.8 and 21.4 mM NaNO$_3$. This decrease in the CCC was concurrent with the increase in the $\zeta$ potential (become less negative) after exposure to UVA and UVB$^{83}$. 

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Similarly, sunlight irradiation resulted in aggregation of polymer coated Ag NMs (e.g. 6 and 25 nm coated with gum Arabic and 25 nm coated with PVP) to different degrees, depending on surface coating and the UV content of the light. The stability of PVP-Ag NMs was generally better than that of Gum Arabic-Ag NMs\textsuperscript{82}. The UV content of the sunlight is identified to be the driving force of NM aggregation, and the strong oscillating dipole-dipole interaction is believed to be the origin of destabilization\textsuperscript{82}. In parallel, Ag NM toxicity was greatly reduced after sunlight irradiation\textsuperscript{82}. The aggregation rate of bared-, citrate-, and PVP-Ag NMs followed the order UV-365 > xenon lamp > UV-254 > dark for the same type of Ag NMs, which indicated that the wavelength and photo-energy of the light irradiation influence Ag NM aggregation kinetics\textsuperscript{77}. The aggregation rate was highest for bare-Ag NMs followed by citrate Ag NMs and PVP Ag NMs.

2.3.3. Behavior of Sterically Stabilized NMs

For sterically-stabilized Ag NMs, there is a wide discrepancy in the reported CCC values ranging from 111.5 mM NaCl to no CCC up to 10 M NaCl, 530 to no CCC up to 10 M NaNO\textsubscript{3}, and 4.9 to No CCC up to 8 M CaCl\textsubscript{2} (Table 2.1). This can be attributed to the surface coverage of Ag NMs by the capping agent molecules, where high capping agent concentrations may result in full coverage and thus full steric stabilization, whereas lower capping agent concentrations may result in partial coverage and thus electrosteric stabilization (e.g. partial steric stabilization of Ag NMs). Evidence supporting this hypothesis can be drawn from studies on other surface coating such as human serum albumin (HSA)\textsuperscript{67} and guar gum\textsuperscript{85}. For instance, increased concentration of human serum albumin (HSA) protein resulted in a reduced attachment efficiency of citrate-coated Ag NM in K\textsubscript{2}SO\textsubscript{4} electrolyte. The attachment efficiency decreased with increasing HSA
concentration until the attachment efficiency reached zero which coincided with the HSA concentration required to form a monolayer on the surface of Ag NMs. They described the attachment efficiency as a function of surface coverage ($\alpha=(1-\omega)^2$, where $\omega$ is the surface coverage) assuming agglomeration between two colliding particles can only occur when two unoccupied surface sites collide. Similarly, increased concentration of guar gum (a natural, neutrally charged, nonionic, water-soluble polysaccharide; 0 to 4.0 g L$^{-1}$) enhanced the stability of zero valent iron NMs (1.5 g L$^{-1}$); that is resulted in decreased aggregate size (ca. from 500 to 200 nm).

Interestingly, the CCC of monovalent electrolytes (NaCl and NaNO$_3$) for PVP-Ag NMs decreases with the increase in zeta potential (Figure 2.5), which can be used to explain the variability in the reported CCC for PVP-Ag NMs. PVP molecules have little to no charge and fully coated Ag NMs have been reported in the literature to have a zeta potential of approximately -10 mV similar to the value reported by El Badawy 2012, which coincides with a full steric stabilization of the NMs and absence of a CCC value. On the other hand, the studies by Zhang 2012, Huynh 2011 and Lin et al reported significantly high zeta potential values, which are likely to be due to partial surface coating of Ag NMs by PVP molecules and thus these particles might be partially charge and partially sterically stabilized, with those studied by Huynh 2011 being more charge stabilized, thus the lower CCC value. This is in good agreement with the concentrations of PVP used to stabilize NMs in these three studies; that is PVP concentrations follow the order Huynh 2011 < Zhang 2012 < El Badawy 2012. Coating zero valent iron NMs with guar gum (a natural, neutrally charged, nonionic, water-soluble polysaccharide; 0.5 g L$^{-1}$) resulted in a nearly neutrally charged NMs at all pHs (2-12) compared to the bare NMs.
which were positively charged at low pHs and negatively charged at high pHs with an isoelectric point at approximately pH 7.0\textsuperscript{85}. These observations highlight the importance of characterizing the surface coverage of NMs by the capping agent.

To further support the hypothesis on the role of surface coverage on zeta potential and ultimately on CCC observations, the zeta potential for in house synthesized cit-Ag NMs coated with variable number of PVP molecules (\textit{e.g.} 4-16) by ligand exchange is presented in Figure 2.5b, and demonstrates the decrease in zeta potential magnitude with the increase in the number of PVP molecules on the surface of Ag NMs.

No CCC value was measured for Gum Arabic coated Ag NMs (GA-Ag NMs) due to steric stabilization\textsuperscript{32}. GA is a polymer with a high molecular weight (5 x 10$^4$ to 8 x 10$^6$ g mol$^{-1}$) with a strong stabilization power\textsuperscript{33,87}. However, this steric stabilization effect disappeared at long experimental times and enhanced NaCl concentrations\textsuperscript{32}.

Clearly, fully sterically stabilized Ag NMs do not undergo aggregation. Thus, the polymer effect is the key factor determining their stability and NM and medium physicochemical properties do not play any role. However, the key question that remains unanswered is the importance of the capping agent following release of NMs to natural waters and their interaction with other medium constituents that may replace the capping agent due to their high affinity to the NM under consideration. For instance, cystine has been shown to impart aggregation of citrate, PVP and PEG-coated Ag NMs, which was attributed to the higher affinity of cystine than the capping agent to Ag NMs\textsuperscript{31}.
2.4. Conclusions

This review highlights the role of Ag NM and medium physicochemical properties in controlling Ag NM aggregation kinetics (α and CCC). We found that, the CCC for Ag NMs increases with the decrease in particle size at a fixed surface (zeta) potential. Otherwise, the CCC might increase with the increase in NM size, or might be independent of NM size, for variable surface (zeta potential), depending on the dependence of NM surface charge on their size. The CCC increase with the decrease in buffer (e.g. phosphate and carbonate) concentration. The CCC for Ag NM decrease with light irradiation due to degradation of surface coating and reduction of the magnitude of surface charge. The CCC is independent of NM concentration for pure electrostatic interactions. However, in the presence of chemical constituents of high affinity to NMs (e.g. Ag NMs in the presence of cystine, carbonate and phosphate anions) that chemisorb on the surface of NMs, the CCC is NM concentration-dependent.

Fully coated, sterically stabilized Ag NMs do not aggregate even in high ionic strength solutions. However, the stability (or CCC) of partially coated Ag NMs decreases with the decrease in surface coverage by the capping agent. Also, sterically stabilized Ag NMs can be destabilized by surface coating replacement, rendering such particles subject to aggregation.

Clearly, the stability of NMs is a complex issue that cannot be described by an individual parameter such as ionic strength, or concentration of NOM, that has been the focus of a significant number of studies in the literature. NM stability is rather determined by a combination of NM and medium physicochemical properties. Chemical interactions of NMs with water constituents such as buffer, anions, cystine, etc. play an equally
important role in controlling NM stability, especially at environmentally relevant NM concentrations (ca. ng to μg L$^{-1}$ range). Therefore, focusing on the physical interactions (e.g. attraction and repulsion) of NM only when taking into account water chemistry in modeling environmental fate and behavior of NMs$^{88}$ might lead to erroneous conclusions. Overall, these findings are important to underpin nanomaterial risk assessment and environmental fate and behavior studies.
Table 2.1. CCC values of Ag NPs in the literature

<table>
<thead>
<tr>
<th>NP concentration (mg L(^{-1}))</th>
<th>Capping agent concentration (mM)</th>
<th>DLS size (nm)</th>
<th>Zeta potential</th>
<th>Buffer concentration</th>
<th>pH</th>
<th>CCC (mM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bared</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.3</td>
<td>--</td>
<td>67 nm</td>
<td>-22</td>
<td>0.01 M NaHCO(_3)/HNO(_3)</td>
<td>7.0</td>
<td>30 NaCl 25 NaNO(_3) 3 Ca(NO(_3))(_2)</td>
<td>48</td>
</tr>
<tr>
<td>1.264</td>
<td>--</td>
<td>82±1.3 nm</td>
<td>-45.8</td>
<td>0.05 mM NaHCO(_3)</td>
<td>7.0</td>
<td>40 NaCl 30 NaNO(_3) 2 CaCl(_2)</td>
<td>66</td>
</tr>
<tr>
<td>1.264</td>
<td>--</td>
<td>82</td>
<td>-45.8*</td>
<td>0.05 mM NaHCO(_3)</td>
<td>7.0</td>
<td>40 NaCl 30 NaNO(_3) 2 CaCl(_2)</td>
<td>67</td>
</tr>
<tr>
<td>0.90</td>
<td>--</td>
<td>21</td>
<td>-31.5 at 1 mM NaNO(_3)*</td>
<td>0.1 mM NaHCO(_3)</td>
<td>6.9-7.2</td>
<td>200 mM NaNO(_3)</td>
<td>68</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-35</td>
<td>No buffer</td>
<td>7</td>
<td>59.2 NaCl</td>
<td>69</td>
</tr>
<tr>
<td><strong>Citrate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.37</td>
<td>0.31</td>
<td>30</td>
<td>-42</td>
<td>No buffer</td>
<td>7.0</td>
<td>86 NaCl 93 NaNO(_3) 127 Na(_2)SO(_4) 1.6 CaCl(_2) 1.7 Ca(NO(_3))(_2) 1.5 CaSO(_4) 1.9 MgCl(_2) 2.2 Mg(_2)SO(_4)</td>
<td>47</td>
</tr>
<tr>
<td>8.8</td>
<td>3.33</td>
<td>58</td>
<td>-39</td>
<td>0.01 M NaHCO(_3)/HNO(_3)</td>
<td>7.0</td>
<td>70 NaCl 70 NaNO(_3) 5 Ca(NO(_3))(_2)</td>
<td>48</td>
</tr>
<tr>
<td>1.264</td>
<td>1</td>
<td>107.5</td>
<td>-26.5 at 1 mM NaNO(_3), or NaCl*</td>
<td>0.05 mM NaHCO(_3)</td>
<td>7.0</td>
<td>40 NaCl 30 NaNO(_3) 2 CaCl(_2)</td>
<td>67</td>
</tr>
<tr>
<td>1.129</td>
<td>0.0013</td>
<td>72.5</td>
<td>-45 at 1 mM NaCl*</td>
<td>0.15 mM NaHCO(_3)</td>
<td>7.0</td>
<td>47.6 NaCl 2.1 CaCl(_2) 2.7 MgCl(_2)</td>
<td>43</td>
</tr>
<tr>
<td>0.125-1</td>
<td>0.3</td>
<td>30</td>
<td>-46.5</td>
<td>No buffer</td>
<td>7.0</td>
<td>16 Cystine at 0.13 mg L(^{-1})</td>
<td>Ag 60 Cystine at 0.25 mg L(^{-1})</td>
</tr>
<tr>
<td>---</td>
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<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>42</td>
<td>-36.9</td>
<td>No buffer</td>
<td>7.0</td>
<td>110 NaCl 1.7 CaCl(_2) 0.00506 AlCl(_3)</td>
<td></td>
</tr>
</tbody>
</table>

### SDS

| 1.264 | 1  | 82  | -42.0 -26.5 at 1 mM NaNO\(_3\) and NaCl* | 0.05 mM NaHCO\(_3\) | 7.0 | 40 NaNO\(_3\) 50 NaCl 2 CaCl\(_2\) |

### Casein

| 11.5 | -- | 58  | -42 | No buffer | NM | 137.6 NaCl 1.6 CaCl\(_2\) |

### Dextrin

| 11.5 | 0.35 wt % | 27.9 | -29 | No buffer | NM | 589.4 NaCl 1.6 CaCl\(_2\) |

### Alginate

| 10.6±0.33 | 4.75 to 9.71 mg L\(^{-1}\) | 33 for Ag\(^{107}\) 35 for Ag\(^{109}\) | -40 for Ag\(^{107}\) -48 for Ag\(^{109}\) | No buffer | 8.0 | 81 NaCl |

### PVP

<p>| 11.5 | 0.35 wt % or 37 mg L(^{-1}) | 29.6 | -22 | No buffer | NM | 1011 NaCl 1.6 CaCl(_2) |
| 15.3 | 0.75 wt% or 75 mg L(^{-1}) | 72  | -10 | 0.01 M NaHCO(_3)/HNO(_3) | 7.0 | No CCC up to 8 M NaNO(_3) and NaCl; and 10 M Ca(NO(_3))(_2) |</p>
<table>
<thead>
<tr>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>0.54</td>
<td>266.67 mg L⁻¹</td>
<td>69.3</td>
<td>-39.0 at 1 mM NaNO₃*</td>
<td>0.1 mM NaHCO₃</td>
<td>6.9-7.2</td>
<td>530 mM NaNO₃</td>
<td>68</td>
</tr>
<tr>
<td>1.1</td>
<td>0.88 mg L⁻¹</td>
<td>66.9</td>
<td>-48 at 1 mM NaCl*</td>
<td>0.15 mM NaHCO₃</td>
<td>7.0</td>
<td>111.5 NaCl 4.9 CaCl₂</td>
<td>43</td>
</tr>
</tbody>
</table>

**Tween**

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.264</td>
<td>10</td>
<td>88</td>
<td>-19 at 1 mM NaNO₃ and NaCl*</td>
<td>0.05 mM NaHCO₃</td>
<td>7.0</td>
<td>&gt;1000 NaNO₃ 500 NaCl 700 CaCl₂</td>
<td>67</td>
</tr>
</tbody>
</table>

**BPEI**

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>8.8</td>
<td>--</td>
<td>95</td>
<td>+40</td>
<td>Using 0.01 M NaHCO₃/HNO₃</td>
<td>7.0</td>
<td>No CCC</td>
<td>48</td>
</tr>
</tbody>
</table>

**Gum Arabic**

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.99</td>
<td>1.05 mg L⁻¹</td>
<td>174.2</td>
<td>-36.5 at 1 mM NaNO₃*</td>
<td>0.1 mM NaHCO₃</td>
<td>6.9-7.2</td>
<td>No CCC up to 1 M NaNO₃</td>
<td>68</td>
</tr>
<tr>
<td>21±1</td>
<td>4.75 to 9.71 mg L⁻¹</td>
<td>55</td>
<td>-42</td>
<td>No buffer</td>
<td>8.0</td>
<td>No CCC for NaCl</td>
<td>71</td>
</tr>
</tbody>
</table>

* Estimated from reported zeta potential based on Smoluchowski assumption
Table 2.2. CCC values of Ag NPs in the literature in the presence of natural organic matter

<table>
<thead>
<tr>
<th>NP concentration (mg L(^{-1}))</th>
<th>Capping agent concentration (mM)</th>
<th>DLS size (nm)</th>
<th>NOM (mg L(^{-1}))</th>
<th>Buffer concentration</th>
<th>pH</th>
<th>CCC (mM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bared</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>100</td>
<td>SRNOM (10)</td>
<td>No buffer</td>
<td>7</td>
<td>47.3 NaCl 3 CaCl(_2)</td>
<td>69</td>
</tr>
<tr>
<td><strong>Citrate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.37</td>
<td>0.31</td>
<td>30</td>
<td>SRFA (5)</td>
<td>No buffer</td>
<td>7.0</td>
<td>152 NaCl 151 NaNO(_3) 76.5 Na(_2)SO(_4) 3 CaCl(_2) 2.4 Ca(NO(_3))(_2)</td>
<td>47</td>
</tr>
<tr>
<td>1.129</td>
<td>0.0013</td>
<td>72.5</td>
<td>SRHA (2)</td>
<td>0.15 mM NaHCO(_3)</td>
<td>7.0</td>
<td>72.1 NaCl 9 CaCl(_2)</td>
<td>43</td>
</tr>
<tr>
<td><strong>Casein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.5</td>
<td>--</td>
<td>58</td>
<td>SRHA (10)</td>
<td>No buffer</td>
<td>NM</td>
<td>2219 NaCl 24.7 CaCl(_2)</td>
<td>70</td>
</tr>
<tr>
<td><strong>Dextrin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.5</td>
<td>0.35 wt %</td>
<td>27.9</td>
<td>SRHA (10)</td>
<td>No buffer</td>
<td>NM</td>
<td>609 NaCl 21.6 CaCl(_2)</td>
<td>70</td>
</tr>
<tr>
<td><strong>PVP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.5</td>
<td>0.35 wt % or 37 mg L(^{-1})</td>
<td>29.6</td>
<td>SRHA (10)</td>
<td>No buffer</td>
<td>NM</td>
<td>3496 NaCl 120 CaCl(_2)</td>
<td>70</td>
</tr>
<tr>
<td>1.1</td>
<td>0.88 mg L(^{-1})</td>
<td>66.9</td>
<td>SRHA (2)</td>
<td>0.15 mM NaHCO(_3)</td>
<td>7.0</td>
<td>155 NaCl 18 CaCl(_2)</td>
<td>43</td>
</tr>
</tbody>
</table>

SRFA: Suwannee River fulvic acid, SRNOM: Suwannee River natural organic matter, SRHA: Suwannee River humic acid, PLFA: pony Lake fulvic acid
Table 2.3. Spearman’s correlation tests between CCC (mM) and Z-average diameter (nm), N-average diameter (nm) and buffer concentration (mM), respectively, under various experimental conditions.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Spearman’s rank correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CCC(mM) vs. Z-average diameter(nm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>-0.7529</td>
<td>0.0075</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>-0.7545</td>
<td>0.0499</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>-0.4928</td>
<td>0.3206</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.2000</td>
<td>0.9167</td>
</tr>
<tr>
<td>NaCl &amp; NaNO$_3$</td>
<td>-0.7785</td>
<td>0.0001</td>
</tr>
<tr>
<td>CaCl$_2$ &amp; Ca(NO$_3$)$_2$</td>
<td>-0.2260</td>
<td>0.5301</td>
</tr>
<tr>
<td><strong>CCC(mM) vs. N-average diameter(nm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>-0.3095</td>
<td>0.3544</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>-0.7106</td>
<td>0.0735</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>-0.6471</td>
<td>0.1649</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.8000</td>
<td>0.3333</td>
</tr>
<tr>
<td>NaCl &amp; NaNO$_3$</td>
<td>-0.4564</td>
<td>0.0569</td>
</tr>
<tr>
<td>CaCl$_2$ &amp; Ca(NO$_3$)$_2$</td>
<td>0.1219</td>
<td>0.7373</td>
</tr>
<tr>
<td><strong>CCC(mM) vs. Buffer concentration(mM)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>-0.7566</td>
<td>0.0070</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>0.1132</td>
<td>0.8089</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>0.3757</td>
<td>0.4629</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.6838</td>
<td>0.3162</td>
</tr>
<tr>
<td>NaCl &amp; NaNO$_3$</td>
<td>-0.4375</td>
<td>0.0694</td>
</tr>
<tr>
<td>CaCl$_2$ &amp; Ca(NO$_3$)$_2$</td>
<td>0.3486</td>
<td>0.3236</td>
</tr>
</tbody>
</table>
Figure 2.1. Dependence of CCC for charge stabilized Ag NMs on (a, b) z-average hydrodynamic diameter, and (c, d) N-average diameter for (a, c) monovalent counter-ions, and (b, d) divalent counter-ions.
**Figure 2.2.** Dependence of the attachment efficiency and CCC on the NM size according to DLVO theory. $A = 3.7 \times 10^{-20}$ J, zeta potential assumed constant 42 mV for all ionic strength conditions for simplification. This figure is used for illustrative purposes and is not meant to be an accurate calculation of the $\alpha$-ionic strength profile.
Figure 2.3. Dependence of secondary minimum depth on NM size according to classical DLVO theory calculated using a Hamaker constant of $3.7 \times 10^{-20}$ J, assuming a constant zeta potential of 42 mV, and IS of 30 mM.
Figure 2.4. Dependence of CCC for charge stabilized Ag NMs on buffer concentration for (a) monovalent counterions, and (b) divalent counter-ions.
Figure 2.5. (a) CCC of PVP coated-Ag NMs as a function of zeta potential of as synthesized PVP-Ag NMs (data collected from the literature). For the highest CCC value, CCC was assumed to be the highest concentration of electrolyte investigated for illustration purposes, but no CCC was measured,48 and (b) zeta potential of citrate-Ag NMs as a function of number of PVP molecules per nm$^2$ surface area, following citrate replacement by PVP using ligand exchange approach (experiments performed by authors).
CHAPTER 3

THE CONCENTRATION-DEPENDENT AGGREGATION OF AG NPS INDUCED BY CYSTINE

Running title: The concentration-dependent aggregation of Ag NPs induced by cystine

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Abstract

Cystine is widely used in cell culture media. Cysteine, the reduced form of cystine, is widely used to scavenge dissolved Ag in eco-toxicological studies to differentiate dissolved vs. nanoparticle uptake and toxicity. However, little is known about the impact of cysteine and cystine on the aggregation behavior of Ag NPs, in particular as a function of Ag NP concentration. Herein, we investigate how cystine (0–300 μM) affects the stability of citrate-, polyvinylpyrrolidone-, and polyethylene glycol-coated silver nanoparticles (cit-Ag NPs, PVP-Ag NPs and PEG-Ag NPs, respectively) with and without Suwannee River fulvic acid (SRFA) as a function of Ag NPs concentration using UV–vis spectroscopy at environmentally and ecotoxicologically relevant Ag NP concentrations (ca. 125–1000 μg L⁻¹). The results demonstrate, for the first time, the concentration-dependent aggregation of cit-Ag NPs in the presence of cystine with a shift in the critical coagulation concentration (CCC) to lower cystine concentrations at lower cit-Ag NP concentrations. At the highest cit-Ag NP concentration (1000 μg L⁻¹), reaction limited aggregation was only observed and no CCC was measured. SRFA slowed the aggregation of cit-Ag NPs by cystine and aggregation occurred in reaction limited aggregation (RLA) regime only. No CCC value was measured in the presence of SRFA. Cystine replaces citrate, PVP and PEG coatings, resulting in aggregation of both electrostatically and sterically stabilized Ag NPs. These findings are important in understanding the factors determining the behavior of Ag
NPs in cell culture media. Also due to the similarity between cystine and cysteine, these results are important in understanding the uptake and toxicity of Ag NPs vs. Ag ions, and suggest that the reduction of the toxicity of Ag NPs in the presence of cysteine could be due to a combined effect of scavenging Ag\(^+\) ions and Ag NP aggregation in the presence of cysteine.

3.1. Introduction

The rapid development of nanotechnology and concern regarding the potential environmental and human health implication of nanoparticles (NPs) has spurred extensive interest in understanding the behavior, fate and effects of NPs.\(^6\) Silver NPs (Ag NPs) are among the most widely used NPs in consumer products as antimicrobial agent.\(^8\) Several studies have suggested that Ag NPs can be released from nano-enabled products and find their way to the environment, resulting in environmental and human exposure to Ag NPs.\(^{12,13}\)

Considerable research has been conducted to better understand the effect of Ag NPs on a wide range of organisms and cell lines\(^{72}\), with a particular attention given to differentiate the NP from dissolved metal effects due to the known solubility of Ag NPs.\(^{18,73-75}\) Several studies have reported mixed results; some show particle-specific toxicity\(^{18,76-78}\), others predominantly relate the effect to free Ag\(^+\) ions\(^{53,79}\), while other studies suggested combined effect of Ag NPs and free Ag\(^+\) ions\(^{80,81}\). These controversies could be partially due to the lack of understanding of NP behavior in the ecotoxicological media\(^{72}\), in particular at ecotoxicologically relevant concentrations.\(^{82,83}\)

To differentiate Ag\(^+\) from Ag NPs effect, binding ligands (e.g. Cl\(^-\) and cysteine) are typically used to complex the released silver ions from Ag NPs\(^{18,81}\), with little
understanding of the impact of the ligands (e.g. cysteine) on the behavior of Ag NPs in the test medium. Several studies have reported reduced toxicity of Ag NP suspension in the presence of cysteine and attributed this reduction solely to the complexation of free Ag$^{+}$ ions by cysteine$^{18, 81}$, without considering the potential destabilization of Ag NPs in the presence of cysteine. Therefore, understanding the interaction between Ag NPs and cysteine is an important issue to underpin the assessment of dissolved silver and Ag NP uptake and toxicity in aquatic organisms. Additionally cystine, the oxidized form of cysteine, is widely used in cell culture media.$^{84}$ Cysteine and cystine are essential amino acids in cell culture media that must be supplied from an exogenous source.

On the other hand, the fate, transport and effects of Ag NPs in the environment depends on their interaction with naturally ubiquitous organic ligands such as thiols (cysteine), disulfide (cystine) and natural organic matter (NOM; fulvic and humic acids), which may chemically, or physically sorb onto NP surface and modify Ag NPs surface properties and colloidal stability.$^{85, 86}$ For example hydrosulfide groups in NOM can form strong covalent bonds with Ag NPs surface and replace the original surface coating such as polyvinylpyrrolidone (PVP)$^{87, 88}$, and thus reduce PVP-Ag NPs stability by increasing ionic strength$^{54}$. Aggregation of NPs (e.g. Ag NPs and Au NPs) can lead to a reduction in NP toxicity.$^{89, 90}$

Few studies have investigated the effect of cysteine on the stability of Ag NPs$^{86}$ and their fate and transport in porous media$^{91}$, typically at high Ag NP concentrations (ca. $>1$ mg L$^{-1}$)$^{72, 85, 86}$, and observed slight aggregation of cit- and PVP-Ag NPs over a period of 48 h. However, there are currently no studies on the impact of cystine on the behavior of Ag NPs, in particular at low NP concentrations.
The aim of this study is therefore to investigate the stability of citrate-, PVP- and PEG-coated Ag NPs following their interaction with cystine. In particular, we investigated the impact of cystine on cit-Ag NP aggregation kinetics at environmentally and ecotoxically relevant Ag NP concentrations (125–1000 μg L\(^{-1}\)), and the impact of SRFA on the interaction between cit-Ag NPs and cystine. We also investigated the aggregation of citrate, PVP and PEG-Ag NPs up to 24 h post interaction with different concentrations of cystine. Cystine (disulfide form of cysteine) and Suwannee River fulvic acid (SRFA) were chosen to represent two distinct types of NOM. Cystine and cysteine represent a class of small molecular weight metal-complexing ligands with a thiol and disulfide groups and are commonly present in surface waters at low concentrations (nM to μM range).\(^{54,92}\)

Cysteine is also widely used in Ag NP toxicity experiments to differentiate the toxicity of NP vs. Ag\(^+\) ions. Cystine rather than cysteine was used in this study for the following reasons 1) the weaker affinity of the disulfide group to Ag\(^+\), thus minimizing the impact on Ag NP dissolution and enabling the use of UV–vis to study the aggregation behavior of Ag NPs, 2) cysteine is highly reactive and readily oxidizes to cystine under oxic conditions\(^{93,94}\), and 3) Cystine is the predominant form in plasma, extracellular body fluids and cell culture media.\(^{95}\) SRFA represent a class of less-defined natural macromolecules, which usually stabilize NPs.\(^{47,82}\)

3.2. Materials and Methods

3.2.1. Chemicals

Silver nitrate (ACS grade, 99.9\(^{+}\)%\) and sodium borohydride (98%) were purchased from Alfa Aesar (Ward Hill, MA, USA). Trisodium citrate (Lab grade) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Cystine (L-cystine ≥98% TLC) was purchased
from Sigma Aldrich (St Louis, MO, USA). SRFA was purchased from the International Humic Substances Society (St Paul, MN, USA). All chemicals were used without further purification.

3.2.2. Synthesis and characterization of silver nanoparticles

Citrate-coated silver NPs (cit-Ag NPs) were synthesized and characterized as described in previous publications. Briefly, cit-Ag NPs were prepared by the reduction of silver nitrate in trisodium citrate. A 100 mL of 0.31 mM trisodium citrate, 100 mL of 0.25 mM silver nitrate and 10 mM of sodium borohydride solutions were prepared in high purity water and kept at 4 °C in the dark for 30 min. The silver nitrate and trisodium citrate solutions were mixed together in a conical flask and vigorously stirred. Subsequently, 6 mL of the reducing agent, sodium borohydride (NaBH₄), was added in one batch. After 10 min of stirring, the solution was heated slowly to boiling and heated for a further 90 min, left overnight and cooled (4 °C, in the dark). Ag NPs were then cleaned, to remove the excess reagents before use, by ultrafiltration (Amicon, 1 kDa regenerated cellulose membrane, Millipore) using a diafiltration method to prevent NP aggregation and drying. Ag NPs were re-dispersed in 0.31 mM trisodium citrate solution to avoid further growth; this process was repeated at least three times. The concentration of Ag NPs was measured by inductively coupled plasma-optical emission spectroscopy and was about 10.0 ± 0.3 mg L⁻¹.

PVP- and PEG-Ag NPs were prepared by ligand exchange approach using citrate-coated Ag NPs as precursor as described elsewhere. Aliquots of cit-Ag NPs were converted into PEG- and PVP-stabilized Ag NPs by adding 8 molecules of PVP, or 4
molecules of PEG per nm$^2$ surface area of cit-Ag NPs under vigorous stirring (e.g. 700 rpm) for at least 1 h and then cooled overnight at 4 °C.

The size and electrophoretic mobility (EPM) was measured by dynamic light scattering and laser Doppler electrophoresis, respectively using a Malvern Zetasizer NanoZS Instrument (Malvern, USA). The Malvern Zeta potential transfer standard consisting of Latex dispersed in pH 9.2 buffer and has an EPM value of $-42$ mV (DTS1235, Malvern Instrument Limited) was used to verify the performance of the instrument and the zeta potential cell throughout the experiments.

The optical absorbance spectrum of Ag NPs were collected by a UV–vis spectrometer (UV-2600 Shimadzu, Santa Clara, CA, USA) using a 100 mm path length cuvette, which allowed analysis of Ag NPs at relatively low concentrations (125 to 1000 $\mu$g L$^{-1}$). UV–vis spectra of cit-Ag NPs suspensions were collected over wavelengths ($\lambda$) 200–900 nm.

3.2.3. Solution Chemistry

The aggregation behavior of cit-Ag NPs was investigated by UV–vis spectroscopy in suspensions containing variable concentration of Ag NPs (125 to 1000 $\mu$g L$^{-1}$; 1.16–9.27 $\mu$M Ag) and cystine (0 to 72 mg L$^{-1}$; 0–300 $\mu$M), in the presence and absence of Suwannee River fulvic acid (SRFA, 1 mg L$^{-1}$). No buffer was used to avoid interferences from ions in the buffer solution. For instance, phosphate anions (a component of the commonly used phosphate buffers) have been shown to induce the aggregation of hematite particles$^{98}$ and titanium dioxide NPs.$^{99}$ The pH was measured throughout the experiment and was in the range 5.5–5.7.
A stock suspension of SRFA was prepared by dissolving 2 mg in 10 mL ultrahigh purity water (UHPW), and the pH was adjusted by adding aliquots NaOH to pH 7.0. The suspension was then filtered (450 nm) to remove any aggregated SRFA molecules. A stock suspension of 333 μM (80 mg L⁻¹) cystine was prepared by dissolving 8 mg of cystine in 100 mL of UHPW. The cystine stock suspension was prepared daily before performing the aggregation experiments to avoid losses of cystine by degradation.⁵⁴

For all experiments performed in the absence of SRFA, an aliquot (125, 250, 500, and 1000 μL) of Ag NPs stock suspension was diluted in UHPW and mixed with different aliquots of cystine to achieve a final volume of 10 mL, and thus NP concentrations of 125, 250, 500 and 1000 μg L⁻¹. For all experiments performed in the presence of SRFA, an aliquot of 2 and 4 mL of Ag NPs stock suspension was mixed with 0.8 and 0.4 mL of SRFA stock suspension and topped with UHPW to achieve a final volume of 8 mL, which resulted in a mixture of 20 and 10 mg L⁻¹ SRFA and concentrations of Ag NPs of 2.5 and 5.0 mg L⁻¹. The mixtures were then left for 24 h to reach equilibrium before performing any aggregation kinetic experiments. Then 0.5 and 1 mL of the mixtures were mixed with cystine and topped with UHPW to 10 mL to achieve a final concentration of Ag NPs of 125 and 500 μg L⁻¹, SRFA of 1 mg L⁻¹, and cystine (0–266 μM).

3.2.4. Aggregation kinetics

To quantitatively measure the aggregation kinetics of cit-Ag NPs by UV–vis, the concentration of individual cit-Ag NPs was monitored over time by performing a time scan of the UV–vis absorbance at λ = 394 nm, corresponding to the specific plasmon resonance peak for the individual Ag NPs. The changes in the UV–vis absorbance λ = 394 nm can be
attributed to NP aggregation or dissolution. Analysis of dissolved Ag concentration by ICP-MS following centrifugal ultrafiltration at 3 kD over 24 h, well beyond the time required to determine NP aggregation kinetics, demonstrated that the differences in cit-Ag NP dissolution in the presence or absence of cystine was not significant. Therefore, the changes in the UV–vis absorbance $\lambda = 394$ nm can be attributed to aggregation of primary Ag NPs. The aggregation rate constant is proportional to rate of change of the UV–vis at $\lambda = 394$ nm ($da_{\lambda=394}/dt$, a refers to UV–vis absorption, Eq. (3.1)) during the early stage aggregation, that is slope of the loss in the UV–vis absorbance at $\lambda = 394$ within the first 30 s after mixing Ag NPs with cystine, which was determined by fitting a linear correlation function.

$$k = \frac{1}{oN} \frac{da_{\lambda=394}}{dt}$$  \hspace{1cm} (3.1)

where N is the initial particle concentration and o is the optical factor.

For colloidal systems, plots of the attachment efficiency ($\alpha = 1/W$, determined according to Eq. (3.2), where W is the stability ratio) against electrolyte concentration at a given experimental conditions can be used to characterize NP aggregation kinetics and suspension stability.

$$\alpha = \frac{1}{W} = \frac{k_{\text{slow}}}{k_{\text{fast}}}$$  \hspace{1cm} (3.2)

where $k_{\text{slow}}$ and $k_{\text{fast}}$ represent the aggregation rate constant under reaction (RLA) and diffusion (DLA) limited aggregation regimes. The RLA regime occurs at counter-ion concentrations below the critical coagulation concentration (CCC), whereas the DLA
occurs at counter-ion concentrations above the CCC. The CCC is frequently used to measure the stability of NP suspensions since it quantifies the minimum concentration of the ion/molecule that is required to completely destabilize the NP suspension. The attachment efficiencies under RLA and DLA regimes were fitted by linear functions and their intersections yield the respective CCC. The aggregation behavior of cit-Ag NPs was also monitored qualitatively by collecting the UV–Vis spectra of Ag NPs 10 min post interaction with different concentrations of cystine over wavelength range 300–900 nm.

The zeta potential of Ag NPs and Ag NPs mixed with cystine were measured by laser Doppler electrophoresis using the same Malvern instrument. The reported zeta potentials and standard deviations were determined from 10 replicates of 50–100 runs each. The performance of the zeta potential cells was verified by measuring the zeta potential of the Malvern Zeta potential transfer standard and the zeta potential cell was replaced when any deviation from the standard value was observed.

The aggregation behavior of cit-, PEG- and PVP-Ag NPs was also monitored qualitatively by collecting the UV–Vis spectra of Ag NPs over wavelength range 300–900 nm at 0, 6 and 24 h post mixing with different concentrations of cystine.

3.3. Result and discussion

3.3.1. Properties of the synthesized Ag NPs

According to the cumulant analysis, the z-dh and polydispersity index of cit-Ag NPs in stock suspension are 16.6 ± 0.2 nm, 0.2 ± 0.1, respectively. Those of PEG-Ag NPs were 19.4 ± 0.3 nm, and 0.29 ± 0.003 and those of PVP-Ag NPs were 21.6 ± 0.3 nm and
0.27 ± 0.005. The zeta potential of cit-Ag NPs, PEG-Ag NPs and PVP-Ag NPs was −46.5 ± 4.5 mV, −28.5 ± 1.4 mV, and −23.6 ± 1.1 mV, respectively. These measurements suggest that cit-, PEG- and PVP-Ag NPs are all well dispersed. The UV–Vis spectra of the cit-Ag NPs, PEG-Ag NPs and PVP-Ag NPs in UHPW water shows a single peak centered at 394 nm, 394 nm, and 395 nm, respectively. Full characterization of the synthesized cit-Ag NPs are provided elsewhere.\textsuperscript{47, 96, 97}

3.3.2. Cystine induced aggregation of cit-Ag NPs

The UV–Vis spectra of cit-Ag NPs, after 10 min of mixing with different concentrations of cystine (Figure 3.1a–d) show a broadening of the UV–vis absorbance peak (centered on 394 nm) of as-synthesized cit-Ag NPs at low cystine concentrations (ca. 20 μM). At higher concentrations of cystine (ca. 33–83 μM), the UV–vis spectra show the formation of a second peak centered on higher wavelengths in the visible and near infrared region (NIR) of the spectrum in the range of 500–650 nm. The increase in cystine concentration results in the decrease in the UV–vis absorbance at 394 nm and the increase of the second peak absorbance at 550–560 nm as a consequence of NP interactions, which indicates the loss of primary cit-Ag NPs by aggregation as observed elsewhere.\textsuperscript{47} Furthermore, at the same concentration of cystine (ca. 33 and 67 μM, Figure 3.2), the second peak is shifted to higher wavelengths at lower NP concentrations compared to higher NP concentrations. The increased width of the second peak at lower NP concentrations is likely to be due to the formation of larger Ag NP aggregates,\textsuperscript{47} which can be attributed to the higher decrease in Ag NP surface charge with the decrease in cit-Ag NPs concentration (Figure 3.3a) at the same concentration of cystine (discussed below).
Under such conditions, more cystine molecules are available for interaction with the Ag surface atoms at lower Ag NP concentrations.

The UV absorbance at $\lambda_{394}$ follows a first order decrease at low cystine concentrations and a more rapid, higher order decrease at high cystine concentrations (Figure 3.4a and b). At the same concentration of cystine (Figure 3.4), the relative loss in the UV–Vis absorbance increase with the decrease in NP concentration, indicating higher removal of primary NPs by more aggregation at lower Ag NPs concentrations. The attachment efficiencies of cit-Ag NPs calculated from the loss of the UV–Vis at $\lambda_{394}$ are plotted in Fig. 3.5a, which generally shows RLA and DLA regimes and suggests that the aggregation kinetic of cit-Ag NPs in the presence of cystine are controlled by electrostatic interactions between the cystine coated Ag NPs (discussion below). The increased cystine concentration results in a decrease in the surface charge of the cit-Ag NPs (Figure 3.3a). In the RLA regime, the cit-Ag NPs are kinetically stabilized by an electrostatic barrier resulting from the double layer repulsion and the van der Waals attractions forces. In the DLA, the surface charge of the cit-Ag NPs is sufficiently reduced by the cystine and the energy barrier is no longer sufficient to kinetically stabilize the particles, and therefore, aggregation process is only driven by diffusion. It was not possible to calculate the attachment efficiency at cit-Ag NP concentration of 1000 $\mu$g L$^{-1}$ as the concentration of cystine, within its limit of solubility, was not sufficient to induce DLA and thus it was not possible to calculate the fast aggregation rate.

At the same cystine concentration and under RLA regime (Figure 3.5a), the attachment efficiency decreases with the increase in Ag NP concentration. This behavior can be attributed to the higher abundance of cystine per surface atom of Ag NPs at lower
NP concentrations, resulting in higher coverage of the NP with cystine and therefore higher reduction in Ag NP surface charge. The CCC as a function of Ag NP concentration increases with the increase in NP concentration (Figure 3.5b), which can be attributed again to the fact that cystine is more efficient in aggregating cit-Ag NPs at lower NP concentrations. This is the first time that cystine-induced concentration-dependent aggregation of cit-Ag NPs is demonstrated.

3.3.3. Effect of cystine on cit-Ag NPs surface charge

Cit-Ag NPs are negatively charged (zeta potential of $-46.5 \pm 4.5$ mV). Figure 3.3a shows the zeta potential of Ag NPs (125–1000 μg L$^{-1}$) as a function of cystine concentration, and shows that, for a fixed NP concentration, the magnitude of zeta potential decrease with the increase in cystine concentration. For a fixed cystine concentration, the magnitude of zeta potential decreases with the decrease in NP concentration. These observations are in agreement with the increased aggregation of Ag NPs at higher cystine concentrations and lower NP concentrations.

High uncertainty was observed in the measured zeta potential at low NP concentrations as laser Doppler electrophoresis requires high NP concentrations, in the range of several mg L$^{-1}$ (Malvern, 2005). Therefore, to further confirm the effect of cystine on the surface charge of cit-Ag NPs, a second set of experiments were performed at higher NP concentrations (1 to 3.5 mg L$^{-1}$) and at fixed cystine concentration (225 μM; Figure 3.3b). Again, the results demonstrate that the magnitude of zeta potential of cit-Ag NPs decreased to lower values with the decrease in NP concentration (Figure 3.3b). Thus, cystine is more efficient in reducing the surface charge of cit-Ag NPs at lower NP
concentrations, which can be attributed to the higher cystine to surface Ag atom ratio at lower NP concentrations and thus the higher coverage of Ag NPs by cystine molecules.

The cystine-induced aggregation and surface charge alteration of cit-Ag NPs can be explained by the chemical interaction of the surface Ag atoms with the disulfide group in the cystine molecule and the zwitterion-type (Zwitterion is a neutral molecule with a positive and a negative electrical charge, though multiple positive and negative charges can be present) surface charge behavior of cystine molecules. It has been demonstrated that similar to the thiol group in cysteine, disulfide groups in cystine can sorb on the surface of Ag NPs. Additionally, Ag NPs have been shown to induce S=S cleavage in cystine at low concentrations, but can keep intact S=S bonds bond at a concentration threshold of about 200 μM, which may explain the similarity in the effect of cystine and cysteine in inducing the aggregation of Ag NPs.

After cystine adsorption on the Ag NPs, the cystine molecule has still two functional groups (carboxylic and amine) to form bonds between particles. The pKa value of the carboxylic and amine groups are 1.96 and 8.18, respectively and the isoelectric pH of cystine is 5.07. The pH of the Ag NPs-cystine mixture was about 5.5–5.7, thus cystine has a negative net charge at the experimental conditions. At this pH, the amine functional groups of cystine are protonated (positively charged, NH₃⁺), whereas the carboxylic groups are deprotonated (negatively charged, COO⁻). Thus, the positively charged amino group in the cystine (−NH₃⁺) on the surface of one NP interacts with the negative charge on the surface of other Ag NP (carboxylic group, COO⁻) through electrostatic attraction, and cause aggregation. Hence, the cystine molecule constitutes a bridge among Ag NPs, thus inducing Ag NPs aggregation.
The concentration-dependent aggregation of Ag NPs in the presence of cystine is due to the ratio between Ag and cystine, and thus the surface coverage of Ag NPs by cystine. At lower Ag NP concentration, more cystine molecules are available to react with the surface of Ag NPs, resulting in a higher surface coverage of Ag NPs by cystine, reduction of surface charge of Ag NPs, and increased aggregation of Ag NPs. This is further supported by the shift to higher wavelengths (approximately 10 nm) in the Plasmon resonance of Ag NPs at lower NP concentrations in the presence of cystine (Figure 3.3c), indicating increased interaction with cystine at low NP concentrations.

3.3.4. Aggregation impacted by fulvic acid

The UV–Vis spectra of cit-Ag NPs 10 min post interaction with cystine in the presence of SRFA show little to no change in the spectra in term of peak broadening or absorbance loss at 394 nm (Figure 3.6a and b), suggesting that SRFA prevents (or reduce) cystine induced aggregation of cit-Ag NPs. The rate of the decay $\lambda_{394}$ is generally slower and decays to higher absorbance in the presence of SRFA ($\sim 0.88$, Figure 3.6a and b) compared to the absence of SRFA ($\sim 0.6$, Figure 3.3a and c), presumably due to the reduced aggregation of cit-Ag NPs in the presence of SRFA. It was not possible to calculate the attachment efficiency in the presence of SRFA because aggregation of cit-Ag NPs did not reach the fast aggregation regime (DLA) within the limit of cystine solubility in water (333 $\mu$M). Nonetheless, the rate loss of UV–Vis$_{394}$ increases slightly with the increase in cystine concentration, but does not reach the fast aggregation rate (Figure 3.7a).

It is well known that SRFA forms a surface coating on cit-Ag NPs and increase the surface charge and provide steric stabilization$^{45, 82, 109, 110}$ to cit-Ag NPs. Therefore, the
sorption of SRFA molecules on cit-Ag NPs increases electrosteric stabilization of cit-Ag NPs, but they do not completely inhibit surface charge screening and NP aggregation by cystine.\textsuperscript{47, 111} It is worth noting that, in the presence of SRFA, the surface charge of cit-Ag NPs did not change with the increase in cystine concentrations (Figure 3.7b), which can be attributed to the lack of interaction between cystine and the surface of Ag NPs. Similarly to the case of without SRFA, another set of experiment was conducted to measure the zeta potential of Ag NPs 24 h post interaction with cystine in the presence of SRFA at higher Ag NP concentrations (1.0–3.5 mg L\(^{-1}\) Ag) to avoid any uncertainty in the measured zeta potential at low NP concentrations (Figure 3.7c). The results suggest that the magnitude of the zeta potential decreases with the decrease in NP concentration at constant cystine concentration (225 μM), indicating the increased efficiency of surface charge alteration at lower NP concentrations due to the increased probability of surface Ag atoms-cystine interactions. Nonetheless, the magnitude of the zeta potential in the presence of SRFA (ca. −28 to −44 mV) remains higher compared with that in the absence of SRFA (ca. −17 to −24 mV), indicating that SRFA form a surface coating on Ag NPs, enhance their surface charge and thus their stability in the presence of cystine. The UV–Vis of Ag NPs spectra 24 h post interaction with cystine in the presence of SRFA shows no shift in the first peak position with the change in NP concentrations (Figure 3.7d) confirming that SRFA limits the interaction of cystine with surface of Ag NPs. Furthermore, Figure 3.7d shows the formation of a second peak at higher wavelengths. For the same concentration of Ag NPs, the absorbance of the second peak is lower in the presence of SRFA (Figure 3.7d) compared with that in the absence of SRFA (Figure 3.3c), again confirming the role of SRFA in reducing the cystine-induced aggregation of Ag NPs.
3.3.5. Aggregation impacted by NP surface coating

It is well known that surface coatings have a significant impact on NP stability and aggregation. Whereas citrate and PEG stabilize Ag NPs via electrostatic repulsion, PVP stabilizes Ag NPs via steric stabilization mechanism.\textsuperscript{27, 47} Therefore, citrate and PEG coated Ag NPs are susceptible to aggregation by charge screening by electrolytes, whereas PVP coated Ag NPs are generally stable at a wide range of ionic strengths and pHs.\textsuperscript{27, 47} Here, we investigate the impact of cystine on the stability of PEG- and PVP-Ag NPs at ecotoxicologically relevant and near environmentally relevant Ag NP concentrations.

The UV–Vis absorbance of cit-, PEG- and PVP-coated Ag NPs (225 $\mu$g L$^{-1}$) immediately after addition of different concentrations of cystine (7–133 $\mu$M) are presented in Figure 3.8a, d and g, together with those after 6 h (Figure 3.8b, e and h) and 24 h (Figure 3.8c, f and i). All spectra show two peaks, the first centered on 394 to 402 nm, 393–400 nm and 393–412.5 nm immediately after addition of cystine for citrate-, PVP, and PEG coated Ag NPs, respectively. The position of the first peak shifts to higher wavelengths at higher concentrations of cystine indicating increased interaction between Ag NP surface atoms and cystine molecules. These observations confirm that cystine replaces the three types of surface coatings (citrate, PVP and PEG). The higher shift at higher cystine concentrations indicates increased interaction between Ag NP surface atoms and cystine molecules.

The first peak shifts to higher wavelength with time; ca. 394–422 nm, 394–415 nm, and 393–424 nm, for citrate-, PVP, and PEG coated Ag NPs 6 h post interactions with cystine. The position of this peak remains constant beyond 6 h up to 24 h, indicating that cystine-Ag NP surface atom interaction reaches equilibrium within 6 h.
The second peak is centered on 495 nm immediately after addition of cystine, shifts to higher wavelengths (ca. 530 nm) between 0 and 6 h and remains constant afterwards. These results indicate that cystine induces the aggregation of citrate, PEG and PVP coated Ag NPs, and that under the selected experimental conditions, Ag NPs occur both as primary Ag NPs as well as aggregated Ag NPs. These findings suggest that cystine binds to the surface of Ag NPs and replaces PVP and PEG coating similar to citrate, resulting in modification of NP surface coating and charge and therefore their aggregation behavior, in good agreement with other studies. The Plasmon resonance peaks was stable within 6 h indicating that Ag NPs were stable at this time point and did not undergo any further aggregation. No losses in the Plasmon resonance were observed at 24 h compared to 6 h indicating that aggregation resulted in the formation of small Ag NP aggregates that did not settle out of suspension. These findings are in good agreement with previous results suggesting that cystine induces aggregation of PVP-coated Ag NPs.

3.3.6. Environmental and nanotoxicological implications

This research represents the first systematic study of the effect of cystine concentration on the aggregation of Ag NPs at ecotoxicologically relevant and near environmentally relevant Ag NP concentrations. Cystine significantly impacts the stability of citrate, PEG and PVP coated Ag NPs, resulting in Ag NP aggregation in a concentration-dependent manner, with a shift in the CCC toward lower concentrations of cystine at lower concentration of Ag NPs. SRFA reduced the cystine induced aggregation of Ag NPs within the limit of cystine solubility in water, likely due to the steric effect of SRFA. Consequently, in natural surface waters, it is likely that cystine will play an important role in determining the fate and behavior of cit-Ag NPs, and further research is required to
investigate the interplay between cystine, electrolytes and NOM on the stability of Ag NPs. Due to the higher affinity of the cysteine thiol groups to Ag\(^{+}\) and Ag surfaces compared with the cystine disulfide group, a similar concentration-dependent aggregation of Ag NPs in the presence of cysteine is expected. Several studies have demonstrated that cysteine induces aggregation of Ag NPs\(^{54}\), but none has investigated the concentration-dependent aggregation of Ag NPs in the presence of cysteine.

In ecotoxicological studies of Ag NPs, cysteine is typically used to scavenge the dissolved ions and thus to differentiate dissolved vs. NP effects assuming no interaction occurs between Ag NPs and cysteine. Several papers have reported lower toxicity of Ag NPs in the presence of cysteine, and consequently concluded that silver ions are the major cause of their antimicrobial activity. Our results suggest that the reduced toxicity in the presence of cysteine widely observed in the literature could be due to a combined effect of Ag\(^{+}\) scavenging by cysteine and Ag NP aggregation. The concentration-dependent aggregation of Ag NPs induced by cystine (a similar behavior is expected with cysteine) can alter the nature of the dose (toxicant) in a typical dose-response experiment; that is Ag NPs may form aggregates at low concentration and stay suspended as individual NPs at high Ag NP concentration.

This work underscores the importance of understanding the impact of biogeochemical molecules on NP stability in both ecotoxicological media as well as in the natural environment, and thus the fate and effects of NPs. This study also underscores the importance of investigating NP interactions at environmentally and toxicologically relevant NP concentration.
Figure 3.1. UV–vis spectra of cit-Ag NP reacted with different concentrations of cystine at different concentrations of cit-Ag NPs: (a) 125 μg L^{-1} Ag, (b) 250 μg L^{-1} Ag, (c) 500 μg L^{-1} Ag, and (d) 1000 μg L^{-1} Ag.
Figure 3.2. UV-Vis spectra at different NP concentrations (μg L⁻¹) at constant cystine concentration (a) 33 μM and (b) 67 μM
Figure 3.3. Zeta potential of cit-Ag NPs reacted with cystine (a) at different concentrations of cystine and cit-Ag NPs, and (b) at fixed concentration of cystine (225 μM) and different concentrations of cit-Ag NPs (1–3.5 mg L\(^{-1}\)). (c) UV–Vis spectra of cit-Ag NPs 24 h post interaction with 225 μM cysteine.
Figure 3.4. Normalized UV-Vis absorbance at 394 nm at different NP concentrations (µg L⁻¹) at constant cystine concentration (a) 33 µM and (b) 67 µM
Figure 3.5. Attachment efficiency of cit-Ag NPs reacted with cystine at different concentrations of cit-Ag NPs.
Figure 3.6. UV-Vis spectra of Ag NP reacted with different concentrations of cystine at different concentrations of Ag NPs in the presence of 1 mgL⁻¹ SRFA: (a) 125 μgL⁻¹ Ag, (b) 500 μgL⁻¹ Ag. Normalized UV-Vis absorbance at 394 nm (c) 125 μgL⁻¹ Ag and (d) 500 μgL⁻¹ Ag
Figure 3.7. Attachment efficiency of cit-Ag NPs reacted with cystine at different concentrations of cit-Ag NPs.
Figure 3.8. UV–vis spectra of Ag NPs (225 μg L$^{-1}$) with different surface coatings (a–c) citrate, (d–f) PVP, and (g–i) PEG at different time points (a, d, g) 0, (b, e, h) 6 and (c, f, i) 24 h following reaction with different concentrations of cystine (7–133 μM)
CHAPTER 4

POTENTIAL IMPACT OF NATURAL ORGANIC LIGANDS ON
THE COLLOIDAL STABILITY OF SILVER NANOPARTICLES

Afshinnia, K.; Marrone, B.; Baalousha, M., Potential impact of natural organic ligands on
the colloidal stability of silver nanoparticles. Science of the Total Environment 2018, 625,
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Abstract

Interaction of natural organic matter (NOM) with engineered nanoparticles (NPs) determine NP fate, transport, and environmental persistence. However, the effect of NOM chemical composition, structure, and concentration on aggregation kinetics and dissolution behavior of silver nanoparticles (Ag NPs) are still poorly understood because of heterogeneity and variability in NOM and Ag NP properties. Here, aggregation behavior of citrate-coated silver nanoparticles (cit-Ag NPs with a z-average diameter of 18 nm) was investigated in the presence of L-cysteine (L-cys) and N-acetyl-L-cysteine (NAL-cys) using UV-Vis spectroscopy. We also investigated the effect of Suwannee River fulvic acid (SRFA) and a NOM isolated from the Yukon River (YRNOM) on the stability of cit-Ag NPs. The dissolution of cit-Ag NPs decreased with increased L-cys and NAL-cys concentration from 0 to 10 µM. The critical coagulation concentration (CCC) of cit-Ag NPs decreased in the presence of L-cys and increased in the presence of NAL-cys. Similarly, L-cys destabilizes cit-Ag NPs in the presence of SRFA. The differences in the stability of cit-Ag NPs in the presence of L-cys and NAL-cys can be attributed to the differences in the functional groups in these two cysteine molecules. L-cys has both negatively charged carboxylic group and a positively charged amine group, resulting in bridging between different particles. NAL-cys is a derivative of cysteine wherein an acetyl
group is attached to the nitrogen atom thus shielding the positive charge on the amine group and therefore eliminating the bridging interaction mechanism. SRFA and YRNOM enhanced the stability of cit-Ag NPs and increased the CCC value to higher counter-ion concentrations. The concentration of SRFA (1-5 mg L\textsuperscript{-1}) did not affect the CCC, whereas the increased concentration of YRNOM increased the CCC of cit-Ag NPs to high Na\textsuperscript{+} concentrations likely due to increased sorption of higher molecular weight compounds on the surface of cit-Ag NPs. The outcome of this study suggests the importance of understanding the molecular properties of NOM (e.g. functional groups and molecular weight) in determining cit-Ag NP environmental behaviors.

4.1. Introduction

The wide use of silver nanoparticles (Ag NPs) in consumer products has recently been a focus of concern due to the potential risk from their release to the environment.\textsuperscript{34, 54, 112-114} Once in the environment, Ag NPs undergo several transformations such as surface coating modifications by organic ligands, aggregation, dissolution and sulfidation.\textsuperscript{28, 46, 54} These transformations will impact NP transport, environmental persistence, reactivity, bioavailability and toxicity to exposed organisms.\textsuperscript{28, 46, 114} NP transformation processes depend on NP physicochemical properties (e.g. size, shape, surface charge, and concentration) and the characteristics of the media (e.g. pH, ionic strength, and presence of natural organic macromolecules).\textsuperscript{34, 38, 46}

NOM is ubiquitous in natural environments and varies in concentration from 0.1 to 10’s of mg L\textsuperscript{-1}, depending on biogeochemical and climatic conditions.\textsuperscript{59-61} NOM is composed of a complex mixture of polyelectrolytic and polyfunctional organic molecules that vary both spatially and temporally in terms of molecular composition, acidity,
molecular weight, structure, and charge density. The main components of NOM are humic substances, polysaccharides, lipids, proteins and other classes of organic molecules. The widely diverse composition and properties of NOM (e.g. N- and S-content, functional groups, molecular weight, hydrophobicity, etc.) will significantly impact the molecular composition of NP surface coating, thereby influencing NP fate, behavior, bioavailability and toxicity.

In waters and sediments, thiols rich organic ligands are commonly present as a component of the natural organic matter (NOM) in nanomolar to micromolar concentrations. Additionally, thiol-containing biomolecules such as L-cysteine (L-cys) and N-acetyl-L-cysteine (NAL-cys) are ubiquitously present in biological media such as tissues, blood and bacterial plasma. The hydrosulfide group in cysteine can form covalent bonds with Ag NPs surface and replace the engineered surface coating such as citrate and polyvinylpyrrolidone (PVP). Cystine, the disulfide form of cysteine, can also modify the surface properties, replace the surface coating and decrease the stability of citrate-coated Ag NPs (cit-Ag NPs). Additionally, cysteine has been widely used in Ag NP toxicity studies to chelate the released Ag ions from Ag NPs and thus to differentiate Ag NP toxicity from that of Ag ions assuming that cysteine does not interact or impact the Ag NP stability.

A survey of literature shows variable effects of cysteine and NOM on Ag NPs stability and dissolution dynamics. Whereas Pokhrel et. al. (2013) suggested that cit-Ag NPs are fairly stable in the presence of cysteine, Yi et. al. (2016) demonstrated that cysteine can change the zeta potential and average diameter of Ag NPs which may influence the stability of Ag NPs in water. Similarly contradictory results has been reported on the effect
of NOM on Ag NPs stability. Where one study reported that 10 mg L\(^{-1}\) of fulvic acid did not have a noticeable effect on Ag NP aggregation kinetics, another study reported an increase in stability (e.g., CCC) in the presence of fulvic acid. These apparently contradictory results could be due to the heterogeneity of NOM and Ag NPs properties.

Numerous studies have addressed the effect of inorganic ligands on Ag NP dissolution and aggregation. However, very little is known on the impact of organic ligands on Ag NP dissolution and aggregation. Therefore, the aim of this study is to assess the effect of two thiol containing organic ligands, L-cys and NAL-cys, and two NOM samples (e.g. Suwannee River fulvic acid; SRFA and Yukon River NOM; YRNOM) on the colloidal stability and dissolution behavior of cit-Ag NPs (500 µg L\(^{-1}\)). These two ligands (e.g. L-cys and NAL-cys) are selected because of their biological importance; they have been widely used in Ag NPs toxicity studies to differentiate between the toxicity of Ag NPs and Ag ions; they possess the same number of N- and S- atoms per molecule, yet they have different ionizable functional groups; and they are simple model compounds that will facilitate our understanding of their role in determining Ag NP environmental and biological behaviors. SRFA was used as a NOM surrogate and because it has been widely used in the literature. Yukon River NOM was used as an example of a more complex NOM compared to SRFA as it was isolated by ultrafiltration and likely to include a wider variety of NOM molecules and therefore likely to have a different impact on Ag NP environmental behaviors.
4.2. Methodology

4.2.1. Synthesis and characterization of Ag NPs

Cit-Ag NPs were synthesized using a previously reported method.\textsuperscript{24, 25, 96} Briefly, solutions of 0.25 mM silver nitrate (99.9\textsuperscript{+}% Alfa Aesar), 0.31 mM trisodium citrate (99.9\textsuperscript{+}% British Drug Houses, BDH) and 10 mM sodium borohydride (98%-Alfa Aesar) were prepared in ultrahigh purity water (UHPW, resistivity = 18.2 MΩ·cm) and were kept at 4 °C in the refrigerator for 30 min. Silver nitrate and sodium citrate solutions were mixed together in a conical flask under continuous stirring condition. Then, 6 mL of the reducing agent, sodium borohydride (NaBH\textsubscript{4}), was added into the batch. After 10 min of stirring, the solution was heated to 100 °C for a further 90 min. The synthesized Ag NPs was stored overnight in dark at room temperature and then cooled to 4 °C. Afterwards, 200 ml Ag NP suspension was cleaned using a pressurized stirred ultrafiltration cell (Amicon, 3 kDa regenerated cellulose membrane, Millipore) to remove the excess reagents before use. During the washing process, Ag NP suspension volume was reduced to 100 mL and then replenished by 100 mL of 0.31 mM trisodium citrate solution. This process was repeated four times.

The concentration of the synthesized cit-Ag NPs was measured by inductively coupled plasma-atomic emission spectroscopy (ICP-OES; Varian 710-ES). The total Ag concentration of the stock suspension was 9.37 ± 0.23 mg L\textsuperscript{−1}. The size and electrophoretic mobility (EPM) of cit-Ag NPs were determined by dynamic light scattering (DLS) and laser Doppler electrophoresis, respectively using a Malvern Zetasizer NanoZS Instrument (Malvern, USA). The Malvern Zeta potential transfer standard (DTS 1235) consisting of a polystyrene latex in aqueous buffer at pH 9 with a zeta potential value of −42 ± 4.2 mV
was used to verify the performance of the instrument and zeta potential cell throughout the experiments. The z-average hydrodynamic diameter and size polydispersity index of synthesized cit-Ag NPs in stock suspension were $18.24 \pm 0.22$ nm and $0.22 \pm 0.01$, respectively. The zeta potential of the synthesized cit-Ag NPs (pH 7.0) was $-41.7 \pm 2.0$ mV. Here, the standard deviation value represents the reliability of the replicate measurements and was determined from 5 replicates of size and 10 replicates of zeta potential. The number average core size measured by transmission electron microscopy (TEM) is approximately 10 nm. The smaller core size measured by TEM is attributed to particle dispersity and average weighing; that is number weighing for core size measured by TEM vs. intensity weighing for z-average hydrodynamic diameter measured by DLS.

4.2.2. Cit-Ag NP behavior by UV-Vis Spectroscopy

The behavior (aggregation, dissolution, and sedimentation) of cit-Ag NPs suspended in different concentrations of L-cys and NAL-cys was monitored over 72 hours using a UV-Vis spectrophotometer (UV-2600 Shimadzu, Santa Clara, CA, USA). All suspensions were buffered at pH 7.0 using 0.1 mM potassium phosphate monobasic. The low K$^+$ concentration from the buffer is unlikely to have any effect on cit-Ag NP stability compared to Na$^+$ due to the higher affinity of Na$^+$ than K$^+$ to the carboxylate groups. Aliquots of cit-Ag NP suspension, and L-cys, NAL-cys, and phosphate buffer stock solutions were mixed together in a total volume of 120 ml prior to analysis by UV-Vis to achieve final concentrations of 500 µg L$^{-1}$ Ag NPs, 0.1 mM phosphate buffer, and different concentrations of L-cys and NAL-cys (0.01-10 µM). Cit-Ag NP concentration of 500 µg L$^{-1}$ Ag was used in this study to enable measurement of Ag NP absorption and absorbance spectra by UV-Vis. Aliquots of Ag NPs-L-cys and Ag NPs-NAL-cys mixtures were
extracted at different time points for UV-Vis analysis, and pH measurement, which varied within a narrow range e.g., 6.6 to 6.8 among the different sampling times.

UV-Vis spectra can provide a lot of information on NP behavior including their aggregation, dissolution and sedimentation. However, results can be convoluted and difficult to interpret if all processes occur at the same time. Decrease in UV-Vis absorbance at λ_{max} indicates particle aggregation and/or dissolution.\textsuperscript{33,47} In the absence of aggregation, dissolution can be assumed to be responsible for the decrease in UV-Vis absorbance at λ_{max}.\textsuperscript{33, 47} Aggregation results in the formation of a second peak at higher wavelength centered on approximately λ_{max} (indicated here as λ_{agg max}) of 500-600 nm which increases initially with the increase in NP aggregation and then decreases with NP aggregate sedimentation. This decrease in λ_{agg max} was used as indication of Ag NP aggregate sedimentation.

In order to further underpin UV-Vis analysis, the dissolved Ag fraction was separated by centrifugal ultrafiltration using Amicon Ultra-4 filtration cartridges at 3 kDa membrane (Millipore). Samples were centrifuged at 4000 g for 20 minutes using an Eppendorf 5810R Centrifuge and the dissolved Ag concentration in the filtrate was measured by ICP-OES.

4.2.3. Aggregation kinetics

Two NOM samples were used in the aggregation kinetic experiment including SRFA (International Humic Substances Society, USA) and dissolved NOM (1 kDa-0.45 μm) isolated from the Yukon River, Alaska, USA using ultrafiltration method.\textsuperscript{128} Stock suspensions of SRFA and YRNOM were prepared by dissolving 2 mg of the NOMs in 10 mL ultrahigh purity water. The NOM suspension pH was adjusted to 7.0 by adding an
appropriate amount of 0.1 or 1 M NaOH. The NOM suspensions were then filtered using a 100 nm syringe filter (Hydrophilic Polyvinylidene Fluoride, Millipore) to remove any aggregated molecules.

A stock solution of 1 M sodium nitrate was freshly prepared to make different concentrations of the monovalent electrolyte. Aliquots of Ag NPs and SRFA or Ag NPs and YRNOM stock suspension were mixed together to obtain mixtures with final concentrations of 500 µg L\(^{-1}\) Ag NPs and 1 mg L\(^{-1}\) NOM. The mixtures were then left for 3 hours in the fridge at 4°C to reach equilibrium before performing the aggregation kinetics’ experiment. Similar to the experiment with NOM, stock solutions of 0.1 mM L-cys and NAL-cys were prepared. In order to prepare different concentrations of L-cys and NAL-cys, aliquots of the cysteine solutions were mixed with Ag NPs for 3 hours prior to the aggregation kinetics’ experiment. For aggregation kinetics analysis, aliquots of each of the prepared NOM-Ag NPs mixtures were added to a mixture of different volumes of buffered sodium nitrate solution and buffered UHPW.

Ag NP aggregation kinetics was performed by UV–Vis according to the method described elsewhere.\(^47\) The aggregation rate constant \((k)\) during the initial stage of the aggregation is proportional to the rate of the loss in the UV–Vis absorbance at the specific Plasmon resonance peak of primary Ag NPs \((\lambda = 394 \text{ nm})\).\(^129\) The slope of the loss in the UV–Vis absorbance at \(\lambda = 394 \text{ nm}\) within the first 30 s of mixing Ag NPs with sodium nitrate was fitted by linear correlation function. The 30 seconds time to measure NPs aggregation kinetics ensures that the reduction in the UV–Vis absorbance is only due to Ag NP aggregation.\(^39,47\) The attachment efficiency \((\alpha)\) versus sodium nitrate concentration, which is used to characterize NP stability is calculated according to Eq. (4.1).\(^67\)
\[ \alpha = \frac{K_{\text{slow}}}{K_{\text{fast}}} \]  

(4.1)

Where \( K_{\text{slow}} \) and \( K_{\text{fast}} \) represent the aggregation rate constants under reaction (RLA) and diffusion (DLA) limited aggregation regimes. \( K_{\text{fast}} \) is the average of all aggregation rate constants measured under DLA regime using the same experimental conditions of NP and buffer concentrations. The RLA regime occurs when electrolyte concentration is below the CCC, where NP interaction is driven by electrostatic energy barrier resulting from electrostatic double layer repulsion forces and van der Waals attractions forces. DLA occurs at electrolyte concentration higher than the CCC, where excess electrolyte ions completely screen the surface energy barriers and aggregation process is only driven by NP diffusion.\(^{39,67}\) The CCC is the minimum concentration of sodium nitrate required to completely screen the NP surface charge. The attachment efficiencies under RLA and DLA regimes were plotted as a function of electrolyte concentration for different concentrations of L-cys, NAL-cys, SRFA and YRNOM, and the mixture of L-cys and SRFA. Attachment efficiencies under slow and fast aggregation regimes were fitted by linear functions and their intersection yields the CCC\(^{48}\). All measurements were repeated 3 times and the means and standard deviations of 3 replicates of attachment efficiencies are presented in the relevant figures.

4.3. Results and Discussion

4.3.1. Effect of L-cys and NAL-cys on cit-Ag NP aggregation and dissolution behavior

Figure 4.1 shows the dissolution of cit-Ag NPs (500 \( \mu \text{g L}^{-1} \)) following the interaction with L-cys and NAL-cys in the presence of 0.1 mM phosphate buffer. The concentration of dissolved Ag increases from \textit{ca.} 70 to 145 \( \mu \text{g L}^{-1} \) in the absence of L-cys and NAL-cys over 72 hours. However, both L-cys and NAL-cys reduce the dissolution of
Ag NPs compared to that in the absence of L-cys and NAL-cys, and the reduction in Ag NP dissolution increases with increased cysteine concentrations. At 0.1 µM L-cys (Figure 4.1a) or NAL-cys (Figure 4.1b), dissolved Ag concentrations increases from 75 to 110 µg L⁻¹, indicating slightly decreased dissolution compared to the absence of L-cys, or NAL-cys. At higher (≥ 1 µM) L-cys and NAL-cys concentrations, dissolved Ag concentration decreased immediately from 70 µg L⁻¹ in the absence of L-cys or NAL-cys to 35 and 5 µg L⁻¹ in the presence of 1 and 10 µM L-cys and NAL-cys concentrations respectively, indicating a significant decrease in Ag NP dissolution. Both L-cys and NAL-cys possess thiol group which interact with silver via a strong covalent (thiolate) bond with a stability constant of 10¹³.⁹¹,¹³⁰ Therefore, L-cys and NAL-cys adsorb onto the NP surface with its thiol group, resulting in the formation of Ag₂S layer on the surface of Ag NPs and thus inhibit the dissolution by preventing the subsequent oxidation of Ag NPs.¹¹³,¹¹⁸ The reduced dissolution of Ag NPs by cysteine has been reported in previous studies.¹¹³,¹¹⁶,¹³¹ For example Sigg et. al. reported a decrease in dissolved Ag concentrations in the presence of 0.5 to 5 µM cysteine over 24 h compared to the absence of L-cys.¹³² However, our results contradict the previous study by Gondikas et. al. in which they observed the increase in dissolved Ag concentration released from cit-Ag NPs in the presence of L-cys.⁵⁴ These apparently contradictory results could be due to the different experimental conditions such as L-cys and total Ag concentration.⁵⁴ The higher cysteine/Ag ratio (400 µM cysteine/8 µM Ag NPs) used in Gondikas et. al. could result in the occurrence of high number of free cysteine molecules in the solution which may act as a sink for dissolved Ag and accelerate their dissolution in a similar manner to the effect of chloride on Ag NP dissolution. At low
Cl/Ag concentration, Cl tends to reduced Ag NP dissolution, whereas at high Cl/Ag concentration, Cl tend to increases Ag NP dissolution.\textsuperscript{133, 134}

The UV-Vis spectra of cit-Ag NPs after mixing with different concentrations of L-cys and NAL-cys in the presence of 0.1 mM phosphate buffer are presented in Figure 4.2a-h. In the absence of cysteine, the primary Ag NPs have a maximum absorbance ($\lambda_{\text{prim max}}$) centered at 394 nm. At the very low concentration of L-cys, 0.01 µM (Figure 4.2a), the UV-Vis absorbance at 394 nm decreased to lower values over the 72 hours period without peak broadening or formation of a second UV-Vis absorbance peak at higher wavelengths, indicating the loss of primary cit-Ag NPs in the presence of L-cys most likely by dissolution. At 0.1 µM L-cys (Figure 4.2c), a loss in the UV-Vis absorbance at 394 nm is observed accompanied by the appearance of a second UV-Vis absorbance peak toward higher wavelengths. This second peak is indicative of NP aggregate formation and is centered on $\lambda_{\text{agg max}} = 560$ nm. At 1 µM L-cys concentration (Figure 4.2e), a similar decrease in the primary NP UV-Vis absorbance was observed as for the 0.1 µM L-cys concentration, however, the maximum aggregate UV-Vis absorbance was centered on shorter wavelength ($ca. \lambda_{\text{agg max}} = 500$ nm), which may attributed to the rapid sedimentation of larger Ag NP aggregates as $\lambda_{\text{agg max}}$ is directly proportional to aggregate size.\textsuperscript{135} This sedimentation behavior is more apparent at the highest L-cys concentration (e.g. 10 mM L-cys, Figure 4.2g), where the UV-Vis spectrum indicates the formation of a shoulder at around 450 to 550 nm immediately after interaction with L-cys, which remained apparent with a decreased intensity up to 5 hours. After 24 hours, the UV-Vis absorbance shifted to smaller wavelength and the primary particle absorbance was centered on $ca. \lambda_{\text{prim max}}$ of 350-360 nm indicating that only small Ag NPs remain in suspension. Sedimentation results
in the removal of large Ag NP aggregates, leaving behind the smallest Ag NPs that remain in suspension and results in absorbance peak around ca. 350-360 nm. The selective aggregation and sedimentation of larger particles can be attributed to the size-dependent stability of Ag NPs. For instance, Afshinnia et al demonstrated that larger NPs have smaller CCC values compared with smaller NPs. Frens (1972) demonstrated that NPs with smaller sizes are more stable against electrolyte coagulation than coarser suspensions. He used this observation to separate larger NPs from a mixture of small and large NPs by selective aggregation of large particles.

UV-Vis spectra in the presence of NAL-cys are presented in Figure 4.2b, d, f, and h. Result shows a slight broadening of the UV-Vis absorbance peak centered at 394 nm with the increase in NAL-cys concentration from 0.01 to 10 µM suggesting limited aggregation (formation of few doublets and triplets) of Ag NPs. The absence of a second peak at higher wavelengths indicates that Ag NP aggregation is less extensive in the presence of NAL-cys compared to the aggregation in the presence of L-cys. The differences in Ag NP aggregation behavior in L-cys and NAL-cys solutions is attributed to the difference in the structure of these two organic molecules. L-cys possesses both carboxylic and amine groups. NAL-cys is a derivative of L-cys where an acetyl group is attached to the nitrogen atom. Therefore, at/near neutral pH values, L-cys forms a dipolar ion “zwitterion”, where the carboxylic group is negatively charged and the amine group is positively charged. The negative charge on the surface of one particle interacts with the positive charge on the surface of another particle inducing Ag NP aggregation through bridging mechanism. On the other hand, at/near neutral pH, NAL-cys possesses only one negative charge, thus resulting in a reduction of cit-Ag NPs surface charge, which
explains the slight aggregation indicated by the broadening of the UV-Vis absorbance of primary Ag NPs. The shielding of the positive charge of the amine group by the acetyl group prevents Ag NP aggregation by preventing bridging mechanism. Additionally, NAL-cys has a higher molecular weight (163.19 g mol\(^{-1}\)) compared with L-cys (121.16 g mol\(^{-1}\)) due to the addition of the acetyl group, which may provide some additional steric stability to Ag NPs. Louie et al (2013) demonstrated that higher molecular weight ligands (NOM) increase NP stability.\(^{138}\)

4.3.2. Effect of L-cys and NAL-cys on cit-Ag NP aggregation kinetics

The impact of cysteine ligands on the stability of cit-Ag NPs in the presence of electrolyte (NaNO\(_3\)) was assessed by studying the aggregation kinetics of cit-Ag NPs in the presence of different concentrations of L-cys and NAL-cys (Figure 4.3 and 4.4). In order to measure the aggregation kinetics’ parameters (e.g. \(\alpha\) and CCC), the loss of UV-Vis absorbance of primary NPs due to the aggregation in the presence of NaNO\(_3\) at 0.01 and 0.1 \(\mu\)M L-cys (Figure 4.3a) and 0.01 \(\mu\)M NAL-cys (Figure 4.3b) was monitored at \(\lambda=394\) nm over 10 minutes and the slope of the loss in UV-Vis absorbance was calculated over the first 30 seconds as described above. The rate of UV-Vis absorbance loss at \(\lambda=394\) nm increased with the increase in NaNO\(_3\) concentration. In general, lower electrolyte concentrations (ca. 50-225 mM NaNO\(_3\), Figure 4.3a) were required to induce aggregation of cit-Ag NPs in the presence of L-cys compared to NAL-cys (ca. 100-350 NaNO\(_3\), Figure 4.3b). In other words, NaNO\(_3\) is more efficient in aggregating cit-Ag NPs in the presence of L-cys compared to NAL-cys.

The attachment efficiencies of cit-Ag NPs as a function of NaNO\(_3\) concentration in the presence of L-cys and NAL-cys are presented in Figure 4.4 with the increase in L-cys
concentration, the attachment efficiency profile shifts toward lower electrolyte concentrations. In other words, at the same electrolyte concentration, the attachment efficiency increases and thus the stability of Ag NPs decreases with the increase L-cys concentration. In the presence of NAL-cys, the attachment efficiency profile shifts toward higher electrolyte concentrations. In other words, at the same electrolyte concentration, the attachment efficiency decreases and thus the stability of Ag NPs increases in the presence of NAL-cys. The CCC values calculated based on the attachment efficiency profiles are reported in Table 4.1. Cit-Ag NPs have a CCC value of 186.4±7.6 mM Na⁺ in the presence of 0.1 mM buffer. The CCC value decreased to 157.0±17.1 and 14.7±2.4 mM Na⁺ in the presence of 0.01 and 0.1 µM L-cys, respectively, in good agreement with previous studies reporting decreased stability of Ag NPs in the presence of L-cys.\textsuperscript{54,85,116} Conversely, the CCC increased to 246.0±22.4 mM Na⁺ in the presence of 0.01 µM NAL-cys. The decrease in CCC with the increase in L-cys concentration can be due to the higher abundance of L-cys molecules per Ag NP surface, resulting in an increased number of L-cys per unit surface area of Ag NPs, thus increased number of positively and negatively charged functional groups that can attract each other and reduce the stability of Ag NPs. The increased stability of cit-Ag NPs following interaction with NAL-cys is because NAL-cysteine replaces citrate coating on the surface of Ag NPs, and most likely induces steric stabilization due to its higher molecular weight compared to L-cys, in addition to electrostatic stabilization due to the carboxylic group.

4.3.3. Effect of SRFA and L-cys on cit-Ag NP aggregation kinetics

In the natural environment, cysteine molecules (or thiol containing organic ligands) occur as part NOM; that is a complex mixture of thousands of organic molecules. Thus,
this section investigates the effect of a mixture of cysteine molecules with SRFA on the stability of cit-Ag NPs in order to illustrate the impact of the structure (e.g. functional groups) of thiol containing molecules (L-cys) on the stability of Ag NPs. The attachment efficiencies of cit-Ag NPs in the presence of SRFA and at different L-cys concentrations are presented in Figure 4.5. In the presence of SRFA alone, the attachment efficiency profile shifts to higher electrolyte concentrations indicating increased stability of Ag NPs. In the presence of SRFA and L-cys, the attachment efficiency profile shifts to lower electrolyte concentrations, with an increased shift at higher L-cys concentration, compared with that in the presence of SRFA alone indicating decreased stability in the presence of L-cys. The CCC shifted to higher counter-ion concentration (245.7±5.8 mM Na⁺) in the presence of 1 mg L⁻¹ SRFA compared to the CCC in the absence of SRFA (186.4 mM Na⁺, Table 4.1). This is in agreement with the consensus that SRFA sorbs on the surface of cit-Ag NPs via ligand exchange mechanism and enhance Ag NP stability via electrostatic mechanism.²⁴ ³⁸ ³⁹ ⁴³ ⁴⁶ ¹³⁹ The CCC shifted to lower counter-ion concentration with the increase in L-cys concentration (217.0±5.6 and 75.6±8.5 mM Na⁺ at 0.01 and 0.1 µM, Figure 4.5 and Table 4.1) compared to the CCC in the presence of SRFA (245.7±5.8 mM Na⁺).

4.3.4. Effect of SRFA and YRNOM on the aggregation kinetics of cit-Ag NPs

Attachment efficiencies of Ag NPs in the presence of NOM (SRFA and YRNOM) are presented in Figure 4.6. At the same NaNO₃ concentration, the attachment efficiency is lower in the presence of NOM suggesting that Ag NPs have fewer tendencies to aggregate in the presence of NOM. The CCC increases in the presence of SRFA and YRNOM (Figure 4.6 and Table 4.1) due to sorption of NOM molecules on the surface of
Ag NPs as discussed above. SRFA and YRNOM stabilized Ag NPs to the same extent (e.g. CCC 245.7±5.8 and 248.0±3.3, respectively) at 1 mg L\(^{-1}\) (Figure 4.6a and Table 4.1). The CCC increased slightly to 259.1 mM Na\(^+\) with the increase in SRFA concentration to 5 mg L\(^{-1}\) (Figure 4.6b and Table 4.1). However, the CCC increased by approximately two-fold to 512.7±12.7 mM Na\(^+\) with the increase in YRNOM concentration to 5 mg L\(^{-1}\) (Figure 4.6b and Table 4.1). We speculate that the differences in the behavior of Ag NPs in the presence of SRFA and YRNOM can be attributed to the differences in NOM isolation protocol. Whereas SRFA were isolated by XAD resin and acidification, YRNOM sample was isolated by ultrafiltration at 1 kDa. Thus, YRNOM is more polydisperse compared to SRFA and contains fulvic and humic acids, as well as other fractions of NOM.

Additionally, the increased YRNOM concentration results in an increase in the molecular weight of the YRNOM molecules sorbed on the surface of Ag NPs due to the selective sorption of higher molecular weight fractions compared with low molecular weight fractions. Analysis of the molecular weight of YRNOM formulas that selectively sorbed (e.g., removed from solution following interaction with Ag NPs) on the surface of Ag NPs by Fourier transform-ion cyclotron-mass spectroscopy suggests that the molecular weight of YRNOM formulas that selectively sorbed on the surface of Ag NPs increased from 669 Da to 716.2 Da with the increase in YRNOM concentration from 1 to 5 mg L\(^{-1}\) in the presence of 4 mg L\(^{-1}\) Ag NPs (data not published yet). This is likely to increase the stability of Ag NPs, in good agreement with previous studies. It is worth mentioning here that the NOM molecular weight determined by FT-ICR-MS is < 1 kDa, in agreement with previous reports of the supramolecular assembly concept.
4.4. **Recommendations and future directions**

NOM has been reported to increase the stability of engineered nanoparticles, and some studies suggested that nitrogen- and sulfur-rich NOM more significantly increase the stability of Ag NPs.\textsuperscript{121} However, the role of N and S atoms in determining NP stability depends on the functional groups containing these atoms. Therefore, understanding the role of molecular formula structure (functional groups) in determining NP stability is essential for predicting the transport and eventual fate of NPs as well as NP bioavailability and toxicity.

In this study, the attachment efficiencies of Ag NPs were measured under different conditions that include SRFA, SRFA mixed with L-cys and NAL-cys, and a NOM extracted by ultrafiltration from the Yukon River. Here we illustrate that, not only the elemental composition, but also that the molecular structure (functional groups) of the organic ligands play a significant role in determining the stability of NPs. Whereas NAL-cys increased the stability of Ag NPs, L-cys decreased the stability of Ag NPs. Additionally, whereas SRFA increased the stability of Ag NPs, addition of a mixture of SRFA and L-cys decreased the stability of Ag NPs. It is worth noting here that both L-cys and NAL-cys contain the same number of N and S atoms per molecule. However, NAL-cys is a derivative of cysteine wherein an acetyl group is attached to the nitrogen atom, thus neutralizing the positive charge on the amine group in the L-cys molecule. This study illustrates the need for a better and more in-depth characterization of NOM at the molecular level to better understand the role of NOM in determining the environmental behavior and effects of NPs. A combination of analytical bulk and molecular level characterization tools such as NMR, FTIR, and ultrahigh resolution-mass spectroscopy techniques such as
Fourier transform-ion cyclotron-mass spectroscopy are likely needed to achieve such in
depth understanding of NOM and its role in modulating NP environmental fate and
behavior.

Both L-cys and NAL-cys has been used in ecotoxicological studies\textsuperscript{114} to scavenge
dissolved ions (\textit{e.g.} Ag). However, no study has considered the cysteine-induced Ag NP
destabilization on Ag NP uptake and toxicity. The decreased stability of Ag NPs in the
presence of L-cys could contribute to the decreased uptake and/or toxicity of Ag NPs
reported in the literature, which should be further investigated in future studies. Given that
both L-cys and NAL-cys equally reduce the dissolution of Ag NPs and that NAL-cys
increases the stability of Ag NPs in electrolyte solution, NAL-cys is recommended as a
better alternative in ecotoxicological studies to scavenge dissolved Ag ions.

Additionally, we illustrate that whereas the concentration of SRFA does not
significantly impact the stability of Ag NPs, the increased concentration of YRNOM
increases the stability of Ag NPs due to the increased selective sorption of higher molecular
weight NOMs on the surface of Ag NPs, which increases the steric stabilization of Ag NPs.
SRFA, extracted by XAD resin, represents a subgroup of NOM characterized by low
molecular weight; whereas YRNOM, extracted by ultrafiltration and thus containing a
mixture of fulvic and humic acids as well as other NOM molecules are more complex and
contain molecular formulas of higher molecular weight, which selectively sorb on the
surface of NPs.\textsuperscript{63} Therefore, this study illustrates that the impact of NOM on NP stability
may also depend on the extraction protocol. Studying both XAD-extracted fractions
together with total NOM fractions “isolated by ultrafiltration” is ideal to better understand
the roles of NOM in determining NP environmental and biological interactions. NOM
isolates are simpler and therefore are better suited for mechanistic understanding of NOM-NP interactions, whereas total NOM samples are more environmentally realistic and will provide better information on the expected environmental behaviors of NPs.

Finally, this work underscores the importance of understanding the impact of NOM molecular properties (e.g. molecular composition and structure, molecular weight, etc.) on NP stability in both ecotoxicological media as well as in the natural environment, and thus the fate and effects of NPs.
Table 4.1. CCC values for cit-Ag NPs in the presence of different natural organic matter

<table>
<thead>
<tr>
<th>Sample</th>
<th>CCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AgNPs + l-cys 0.1 μM</td>
<td>14.7 ± 2.4</td>
</tr>
<tr>
<td>AgNPs + SRFA + l-cys 0.1 μM</td>
<td>75.6 ± 8.5</td>
</tr>
<tr>
<td>AgNPs + l-cys 0.01 μM</td>
<td>157.0 ± 17.1</td>
</tr>
<tr>
<td>Ag alone</td>
<td>186.4 ± 7.6</td>
</tr>
<tr>
<td>AgNPs + SRFA + l-cys 0.01 μM</td>
<td>217.0 ± 5.6</td>
</tr>
<tr>
<td>AgNPs + SRFA 1 mg L&quot;⁻¹&quot;</td>
<td>245.7 ± 5.8</td>
</tr>
<tr>
<td>AgNPs + NAL-cys</td>
<td>246.0 ± 22.4</td>
</tr>
<tr>
<td>AgNPs + YRONOM 1 mg L&quot;⁻¹&quot;</td>
<td>248.0 ± 3.3</td>
</tr>
<tr>
<td>AgNPs + SRFA 5 mg L&quot;⁻¹&quot;</td>
<td>259.1³</td>
</tr>
<tr>
<td>AgNPs + YRONOM 5 mg L&quot;⁻¹&quot;</td>
<td>512.7 ± 12.7</td>
</tr>
</tbody>
</table>

³ Only one replicate was performed here.
Figure 4.1. Dissolution of cit-Ag NPs (500 μg L$^{-1}$) following the interaction with different concentrations of (a) L-cys, and (b) NAL-cys. All suspensions were buffered with 0.1 mM potassium phosphate monobasic buffer. Dashed lines are used to guide the readers' eyes.
Figure 4.2. Dissolution of cit-Ag NPs (500 μg L\(^{-1}\)) following the interaction with different concentrations of (a) L-cys, and (b) NAL-cys. All suspensions were buffered with 0.1 mM potassium phosphate monobasic buffer. Dashed lines are used to guide the readers' eyes.
Figure 4.3. Evolution of the UV–vis absorbance at $\lambda_{394}$ of cit-Ag NPs (500 μg L$^{-1}$) as a function of time immediately after mixing with different concentrations of NaNO$_3$ at 0.1 mM phosphate buffer (pH 7.0): (a) 0.01 μM L-cys, and (b) 0.01 μM NAL-cys.
Figure 4.4. Attachment efficiency of cit-Ag NPs (500 μg L$^{-1}$) in the presence of L-cys and NAL-cys. Dashed lines are used to guide the readers’ eye.
Figure 4.5. Attachment efficiency of cit-Ag NPs (500 μg L$^{-1}$) in the presence of L-cys and 1 mg L$^{-1}$ SRFA. Dashed lines are used to guide the readers' eye.
Figure 4.6. Attachment efficiency of cit-Ag NPs (500 μg L$^{-1}$) (a) in the presence of 1 mg L$^{-1}$ SRFA and YRNOM, and (b) in the presence of 5 mg L$^{-1}$ SRFA and YRNOM. Dashed lines are used to guide the readers’ eye.
CHAPTER 5

EFFECT OF PHOSPHATE BUFFER ON AGGREGATION KINETICS OF CITRATE-COATED SILVER NANOPARTICLES INDUCED BY MONOVALENT AND DIVALENT ELECTROLYTES

**Running title:** Effect of phosphate buffer on aggregation kinetics of citrate-coated silver nanoparticles induced by monovalent and divalent electrolytes

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**Abstract**

The attachment efficiency ($\alpha$) is an important parameter that can be used to characterize nanoparticle (NPs) aggregation behavior and has been a topic of discussion of several papers in the past few years. The importance of $\alpha$ is because it is one of the key parameters that can be used to model NP environmental fate and behavior. This study uses UV-Vis and laser Doppler electrophoresis to monitor the aggregation behavior of citrate-coated silver nanoparticles (cit-Ag NPs) induced by Na$^+$ and Ca$^{2+}$ as counter-ions in the presence and absence of Suwannee River fulvic acid (SRFA) as a surrogate of natural organic matter and different concentrations of phosphate buffer (0-1 mM). Results demonstrate that phosphate buffer, which serves to maintain pH nearly constant over the course of a reaction, is an important determinant of NP aggregation behavior. Increasing phosphate buffer concentration results in a decrease in the critical coagulation concentrations (CCC) of cit-Ag NPs to lower counter-ion concentration and an increase of $\alpha$ at the same counter-ion concentration, both in the absence and presence of SRFA. SRFA stabilizes Ag NPs and increases the CCC to higher counter-ion concentrations.

The outcome of this study can be used to rationalize the variation in $\alpha$ and CCC values reported in the literature for NPs with similar physicochemical properties, where different $\alpha$ and CCC values are reported when different types of buffers and buffer concentrations are used in different studies.
5.1. Introduction

Silver nanoparticles (Ag NPs) are nowadays used in a large number of commercial products due to their antimicrobial properties\textsuperscript{139}, resulting in release of Ag NPs to the environment\textsuperscript{12, 13}, with estimated surface water concentrations in the ng to µg L\textsuperscript{-1} range.\textsuperscript{145, 146} Once in the environment, Ag NPs undergo several transformations such as dissolution\textsuperscript{113}, aggregation\textsuperscript{47}, sulfidation\textsuperscript{147}, photochemical and biological transformations.\textsuperscript{30, 148, 149} These transformations of Ag NPs can impact their fate and effects in the environment.\textsuperscript{30, 148, 149} NPs aggregation is one of the key processes determining their reactivity\textsuperscript{6, 56, 65}, bioavailability\textsuperscript{35, 150}, toxicity\textsuperscript{36, 37, 151, 152} and environmental fate and effects.\textsuperscript{35}

Silver nanoparticle aggregation is governed by the physicochemical properties of the NPs (e.g. size, shape, surface charge, and concentration) and the media (e.g. pH, ionic strength, counter-ion valency, and presence of natural organic macromolecules).\textsuperscript{47, 153, 154} NP suspension stability is typically characterized by two fundamental parameters (attachment efficiency, $\alpha$; and critical coagulation concentration, CCC). The attachment efficiency reflects the fraction of NP collisions leading to attachment of two NPs and is $<\ 1.0$ under reaction limited aggregation regime (RLA) and equals 1 under diffusion limited aggregation regime (DLA).\textsuperscript{48, 155} The Critical coagulation concentration (CCC) is the minimum concentration of counter-ions required to completely destabilize the NP suspension. The attachment efficiency ($\alpha$) has been recently a focus of discussion due to its importance to construct NP fate models.\textsuperscript{35, 156} Thus, the recent spike in publications of empirically determined $\alpha$’s or related parameters (e.g. Debye length) for use in such
Nonetheless, there is still a need to better understand the factors determining $\alpha$, among which NP and medium physicochemical properties\textsuperscript{29, 30}.

Buffering NP suspensions to maintain a constant pH, when investigating environmental fate, behavior and effects of NPs is of utmost important to minimize confounding results due to changes in pH in case of the absence of buffer. Several studies have demonstrated that suspension pH affect NP surface charge and aggregation behavior\textsuperscript{29, 55, 56, 82, 158-161}.

For instance, Stemig \textit{et al}. (2014) reported an increase in goethite NP aggregate size and a decrease in their zeta potential with the increased concentration of MOPS (3-Morpholinopropane-1-sulfonic acid) buffer\textsuperscript{56}. Several studies have investigated the aggregation kinetics of Ag NPs; some studies used buffer such as carbonate buffers to maintain a stable pH\textsuperscript{43, 66, 67}, while others did not use any buffer to prevent interferences due to the presence of the buffer\textsuperscript{47, 162}. These studies have reported significant variability in the measured $\alpha$ and CCC values for citrate-Ag NPs, which were attributed to differences in experimental conditions such as media composition (\textit{e.g.} buffer type and concentration) and physicochemical properties of the particles such as particle size and surface coverage of the citrate molecules on the NPs\textsuperscript{162, 163}. However, none of these studies have systematically investigated the effect of buffer type and concentration on the aggregation behavior of Ag NPs.

Therefore, the aim of this study is to assess the effect of phosphate buffer on the stability of citrate-Ag NPs. The stability of Ag NPs was quantified by measuring Ag NP aggregation kinetics using UV-Vis and determining the $\alpha$ and CCC in the presence of different concentrations of potassium phosphate monobasic (0 to 1 mM) as a representative buffer, sodium nitrate and calcium nitrate as monovalent and divalent electrolytes, and
Suwannee River fulvic acid (SRFA) as a surrogate of natural organic matter (NOM). Here we use near environmentally-relevant Ag NPs concentration (e.g. 270.25 µg L\(^{-1}\)). The concentration of Ag NPs used in this study is less than those typically used in aggregation kinetic studies in the literature.\(^{162}\) However, it is still higher than those estimated to occur in environmental systems based on exposure modeling approaches.\(^{145,164}\) Phosphate buffer was used as an environmentally and biologically relevant buffer in this study.\(^{165}\) Phosphate is commonly found in environmental\(^{166}\) and biological and toxicological media.\(^{167-169}\) In the human body, inorganic phosphate concentration ranges from 0.2 to 0.4 mM and from 23 µM to 1 mM in human blood and sweat, respectively. Water bodies, such as rivers and lakes, may contain phosphate ion concentrations of 0.01 to 1 mM, although occasionally at higher concentrations following phosphorous pollution events and in wastewater effluent.\(^{170,171}\) With phosphate’s significant presence in biological systems and in the environment, we pose the question as to whether the phosphate anions can have any influence on Ag NP aggregation kinetics, and hence, the environmental fate, behavior and impact of Ag NPs.

5.2. Methodology

5.2.1. Synthesis and characterization of silver nanoparticles

Citrate-coated silver nanoparticles (cit-Ag NPs) were synthesized and characterized using a previously reported method.\(^{96}\) Briefly, solutions of 100 ml silver nitrate (0.25 mM, 99.9\(^{+}\)% , Alfa Aesar ), 100 ml trisodium citrate (0.31 mM, 99.9\(^{+}\)% , British Drug Houses, BDH) and sodium borohydride (10 mM, 98%-Alfa Aesar) were prepared in ultrahigh purity water (resistivity = 18.2 MΩ.cm) and were then kept at 4\(^{\circ}\)C in the dark for 30 min. Silver nitrate and sodium citrate solutions were mixed together in a
conical flask under continuous stirring condition. Afterward, 6 ml of the solution of the reducing agent, sodium borohydride (NaBH₄), was added in one batch. After 10 min of stirring, the solution was heated slowly to boiling and then heated for a further 90 min. The synthesized Ag NP suspension was then stored in dark overnight and cooled to 4°C. 200 ml of Ag NP suspension was cleaned by pressurized stirred ultrafiltration cell (Amicon, 1 kDa regenerated cellulose membrane, Millipore) to remove the excess reagents before use. Ag NP suspension volume was reduced to 100 ml and then replenished by 100 ml of 0.31 mM trisodium citrate solution. This process was repeated at least three times to avoid further NP growth.

The concentration of the synthesized Ag NPs was measured by inductively coupled plasma-atomic emission spectroscopy (ICP-OES). The total Ag Concentration in the stock suspension was 10.81 mgL⁻¹. The size and electrophoretic mobility (EPM) of Ag NPs were measured by dynamic light scattering (DLS) and laser Doppler electrophoresis, respectively using a Malvern Zetasizer NanoZS Instrument (Malvern, USA). The Malvern Zeta potential transfer standard (DTS 1235) consisting of a polystyrene latex in aqueous buffer at pH 9 with a zeta potential value of -42 ± 4.2 mv was used to verify the performance of the instrument and zeta potential cell throughout the experiments. According to DLS, the z-average hydrodynamic diameter and size polydispersity index of synthesized citrate-Ag NPs in stock suspension were 24.5 ± 0.1 nm and 0.21 ± 0.05, respectively, indicating a narrow size distribution. The zeta potential of the synthesized cit-Ag NPs (pH 7.0) was -41.7 ± 2.0 using Smoluchowski approximation. The standard deviation reported here shows the stability of the replicate measurements and was determined from 5 replicates of size and 10 replicates of zeta potential.
The optical absorbance spectrum of cit-Ag NPs was collected by a UV-Vis spectrophotometer. All spectra were collected using a 100 mm path length cuvette, which allows analysis of Ag NPs at relatively low concentration (270.25 μg L$^{-1}$). The UV-Vis spectrum of Ag NP stock suspension (10.81 mg L$^{-1}$) over wavelength 200-900 nm measured using a 10 mm path length cuvette shows a single peak centered at 396 nm.

5.2.2. Solution Chemistry

The effect of phosphate buffer on the aggregation kinetics of cit-Ag NPs at pH 7 was investigated using UV-Vis spectroscopy by varying the buffer concentration (0-1 mM) in suspensions with different concentration of NaNO$_3$ (99+%, Sigma Aldrich) and Ca(NO$_3$)$_2$ (99+% Alfa Aesar). First, a stock solution of 5 mM potassium phosphate monobasic (99.9+%, BDH) was prepared and the pH was adjusted to 7.0 by adding 2 ml of 1 M NaOH in 1 Liter of 5 mM buffer. Then, the buffer stock solution was diluted to achieve different concentration of buffers (0-1 mM). The final concentration of K$^+$ and Na$^+$ added due to buffer solution and pH adjustment by NaOH are very small ca. < 0.4 and 1mM, respectively. Therefore, any differences in Ag NPs aggregation kinetics, is due to the variation in phosphate buffer concentration (see detailed discussion below). 1 M NaNO$_3$ and 3 mM Ca(NO$_3$)$_2$ electrolyte solutions were prepared in ultrahigh purity water or diluted buffer. Aliquots (250 μl) of Ag NPs were diluted just before performing the aggregation kinetic experiment by adding ultrahigh purity water or buffer and different volumes of electrolytes (1 M of NaNO$_3$, and 3 mM of Ca(NO$_3$)$_2$) to achieve a final volume of 10 mL for UV–Vis experiment. These dilutions resulted in a final concentration of 270.25 μg L$^{-1}$ Ag NPs, and different concentration of electrolytes (40-260 mM NaNO$_3$ and 0.045-1 mM
Ca(NO$_3$)$_2$) for aggregation kinetics analysis using UV-Vis. The pH was measured at the end of each aggregation kinetic experiment and was in the range of 6.7 to 7.0 for buffer concentrations 0.25 to 1 mM and in the range of 6-7 for buffer concentration of 0.05 mM as well as for no buffer.

In order to account for the effect of calcium phosphate precipitation on the concentration of Ca$^{2+}$ ions in solution and thus on Ag NP aggregation kinetics, the speciation of calcium in solutions with different phosphate buffer concentrations was modeled using visual MINTEQ speciation model. Visual MINTEQ is an equilibrium speciation model that can be used to calculate the equilibrium composition of dilute aqueous solutions. Input molar concentration for each component (Ca$^{2+}$, NO$_3^-$, K$^+$, PO$_4^{3-}$) is based on the concentration of these components in the aggregation kinetics experiment. The solution pH was maintained constant at 7.0 and hydroxyapatite was introduced as a possible solid phase.

A stock suspension of SRFA (International Humic Substances Society, St. Paul, MN, USA) was prepared by dissolving 2 mg of SRFA in 10 mL ultrahigh purity water, and the pH was adjusted to 7.0 by adding an appropriate amount of NaOH. The suspension was then filtered through a 100 nm filter to remove any aggregated SRFA molecules. In order to prepare Ag NP suspensions at the same concentrations (e.g. 270.25 μg L$^{-1}$) as the experiments without SRFA, 0.8 mL of the stock suspension of SRFA was mixed with 2 mL of the stock suspension of the Ag NPs and 3.2 mL of ultrahigh purity water giving a mixture of 5.4 mg L$^{-1}$ Ag NPs and 20 mg L$^{-1}$ of SRFA. Then the mixture was left for 24 h to reach equilibrium before performing any aggregation kinetic experiment. Just before performing the experiment, the mixture of Ag NPs and SRFA was diluted by adding
specific amount of buffer and different volume of electrolyte to reach a final volume of 10 ml for UV-Vis analysis. These dilutions resulted in a suspension of 270.25 µg L\(^{-1}\) Ag NPs, 1 mg L\(^{-1}\) SRFA and different concentration of electrolytes for aggregation kinetic analysis by UV-Vis.

Additionally, the effect of phosphate buffer on the aggregation behavior of cit-Ag NPs was investigated by measuring the zeta potential of Ag NPs (5.4 mgL\(^{-1}\)) in the presence of different concentrations of buffer (0-2.5 mM). First, a stock solution of 9 mM potassium phosphate monobasic was prepared and the pH was adjusted to 7.0 by adding aliquot NaOH. Then, buffer stock solution was diluted to achieve different concentrations of buffer (0-5 mM). Aliquots (2.5 ml) of Ag NP stock suspension (10.81 mg L\(^{-1}\)) were diluted in 2.5 ml buffer (0-5 mM) to achieve a final concentration of 5.40 mg L\(^{-1}\) Ag NPs, and different concentrations of phosphate buffer (0-2.5 mM).

5.2.3. Aggregation kinetics

Ag NP aggregation kinetics was performed by UV-Vis according to the method described by Baalousha et al, (2013). Briefly, the aggregation rate constant (k) during the early stage of the aggregation is proportional to the rate of the decrease in the UV-Vis at the specific plasmon resonance peak (λ = 396 nm) of individual Ag NPs.\(^{129}\) The slope of the loss in the UV-Vis absorbance at λ = 396 nm within the first 10 s after mixing of Ag NPs with the electrolytes was determined by fitting a linear correlation function. The short time (10 sec) required to measure NM aggregation kinetics ensures that the reduction in the UV-Vis absorbance is due to Ag NP aggregation only as demonstrated in previous studies.\(^{39,47}\) The attachment efficiency (α) or the inverse stability ratio (W) were calculated according to Eq. 5.1.
\[ \alpha = \frac{1}{W} = \frac{K_{\text{slow}}}{K_{\text{fast}}} \]  
(Eq. 5.1)

Where \( K_{\text{slow}} \) and \( K_{\text{fast}} \) are the aggregation rate constant under reaction (RLA) and diffusion (DLA) limited aggregation regimes. \( K_{\text{fast}} \) is calculated as the average of all aggregation rate constants measured under DLA regime under the same experimental conditions of NP and buffer concentrations. The RLA regime occurs when ionic strength is below the CCC, where NP interaction is governed by electrostatic energy barrier resulting from electrostatic double layer repulsion forces and van der Waals attractions forces. DLA occurs at ionic strength greater than CCC, where excess counter-ions completely screen the surface charge and aggregation process is only driven by NP diffusion. The CCC is the minimum concentration of electrolyte particularly sodium nitrate and calcium nitrate in this study, that is required to completely screen the NP surface charge. The attachment efficiencies under RLA and DLA regimes were plotted as a function of electrolyte concentration for different concentrations of buffer. Then, attachment efficiencies under RLA and DLA regimes were fitted by linear functions and their intersections represent the respective CCC, which is used in this study as a measure of NPs stability.

5.3. Results and Discussion

5.3.1. Impact of phosphate buffer on the aggregation behavior of cit-Ag NPs

The UV-Vis spectra of cit-Ag NPs, after 10 min of mixing with different concentrations of NaNO\(_3\) buffered with different concentration of phosphate buffer (0.05 and 1.0 mM) are presented in Figure 5.1a and b, respectively (full data set at 0, 0.05, 0.25, 0.5 and 1 mM phosphate buffer concentrations is presented in Figure 5.2a-e). At low buffer concentration (0.05 mM, Figure 5.1a), the increase in NaNO\(_3\) concentration results in the
decrease in the UV-Vis absorbance at 396 nm, broadening of the UV-Vis absorbance peak of the as-synthesized cit-Ag NPs (centered on 396 nm), and the formation of a second absorbance peak centered on ca. 600 nm. These changes in the UV-Vis spectra of cit-Ag NPs indicates the loss of primary cit-Ag NPs by aggregation in the presence of NaNO₃ as observed elsewhere. It is well-known that cit-Ag NPs are electrostatically stabilized, and that the increase in counter-ion concentration (here Na⁺) results in surface charge screening, allowing Ag NPs to stick together and aggregate.

At high buffer concentrations (1 mM, Figure 5.1b), a similar loss in the UV-Vis absorbance at 396 is observed together with a formation of a broad second peak that extends to higher wavelengths compared with those at lower buffer concentrations (Figure 5.1a), potentially due to the formation of larger Ag NP aggregates that absorb and scatter light at higher wavelengths. Furthermore, higher concentration of NaNO₃ (ca. 100-260 mM, Figure 5.1a) was required to induce aggregation of cit-Ag NPs at lower buffer concentration (0.05 mM) compared to those (40-100 mM NaNO₃, Figure 5.1b) required to induce aggregation at higher buffer concentration. In general, the increase in buffer concentration in 0-1 mM range (Figure 5.2a-e) results in increased loss of the UV-Vis absorbance at 396 nm and further broadening of the UV-Vis to higher wavelengths indicating increased aggregation of Ag NPs with the increase in buffer concentration.

At the same concentration of NaNO₃ (ca. 100 mM, Figure 5.3a), the loss in the UV-Vis absorbance at 396 nm increases with the increase in buffer concentration, which is accompanied by an increased broadening of the second peak toward higher wavelengths, indicating higher removal of primary NPs by increased aggregation at higher buffer concentrations. In other words, NaNO₃ is more efficient in aggregating cit-Ag NPs at
higher buffer concentration than at lower buffer concentration. The increased cit-Ag NPs aggregation with the increased phosphate buffer concentration is presumably due to the sorption of phosphate anions on the surface of Ag NPs\textsuperscript{172}, resulting in increased reduction of Ag NP surface charge at higher buffer concentrations (see discussion below).

The UV-Vis spectra of cit-Ag NPs after 10 min of mixing with Ca(NO\textsubscript{3})\textsubscript{2} in different concentration of phosphate buffer (0.05 and 1.0 mM) are presented in Figure 5.1c and d, respectively (full data set at 0, 0.05, 0.25, 0.5 and 1 mM phosphate buffer concentrations is presented in Figure 5.4a-e). Similarly to NaNO\textsubscript{3}, the increase in Ca(NO\textsubscript{3})\textsubscript{2} results in increased loss of the UV-Vis absorbance at 396 nm, broadening of the absorbance peak of the as-synthesized cit-Ag NPs, and the formation of a second peak centered on \textit{ca.} 550 nm, indicating the increased aggregation of cit-Ag NPs with the increase in Ca(NO\textsubscript{3})\textsubscript{2} concentration. Furthermore, higher concentration of Ca(NO\textsubscript{3})\textsubscript{2} (\textit{ca.} 0.25-0.8 mM, Figure 5.1c) was required to induce aggregation of cit-Ag NPs at lower buffer concentration (0.05 mM) compared to higher buffer concentration (0.045-0.25 mM Ca(NO\textsubscript{3})\textsubscript{2}, Figure 5.1d). In general, the increase in buffer concentration in 0-1 mM range (Figure 5.4a-e) results in increased loss of the UV-Vis absorbance at 396 nm and further broadening of the UV-Vis absorbance to higher wavelengths indicating increased aggregation of Ag NPs with the increase in buffer concentration.

At the same concentration of Ca(NO\textsubscript{3})\textsubscript{2} (\textit{ca.} 0.25 mM, Figure 5.3b), the loss in the UV-Vis absorbance at 396 nm increases with the increase in buffer concentration, which is accompanied by an increased broadening of the second peak toward higher wavelengths, indicating higher removal of primary Ag NPs by increased aggregation at higher buffer
concentrations. In other words, Ca(NO$_3$)$_2$ is more efficient in aggregating cit-Ag NPs at higher buffer concentration (Figure 5.3b) than at lower buffer concentration.

To measure Ag NP aggregation kinetics’ parameters (e.g. $\alpha$ and CCC), the loss of primary particles due to aggregation in the presence of NaNO$_3$ and Ca(NO$_3$)$_2$ at different buffer concentrations was monitored by measuring the UV-Vis absorbance at $\lambda = 396$ (Figure 5.5, full data set of all different buffer concentrations is presented in Figure 5.6 and 5.7). The UV absorbance at $\lambda=396$ nm follows a first order decrease at low electrolyte concentrations and a more rapid, higher order decrease at high electrolyte concentrations (Figure 5.5a-d). In general, lower electrolyte concentrations (ca. 40-120 NaNO$_3$, Figure 5.5b and 0.045-0.25 mM Ca(NO$_3$)$_2$, Figure 5.5d) were required to induce aggregation of cit-Ag NPs at higher buffer concentration compared to lower buffer concentration (ca. 50-260 NaNO$_3$, Figure 5.5a and 0.25-0.9 mM Ca(NO$_3$)$_2$, Figure 5.5c).

The attachment efficiencies of cit-Ag NPs as a function of initial electrolyte concentration (NaNO$_3$ and Ca(NO$_3$)$_2$) in the presence of different buffer concentrations are presented in Figure 5.8a and b, respectively. In the presence of phosphate anions, calcium precipitates forming hydroxyapatite resulting in a reduction of free calcium ions (Figure 5.9) available to induce Ag NP aggregation. Under the same initial Ca(NO$_3$)$_2$ concentration, the concentration of Ca$^{2+}$ remaining in solution decreases with the increase in buffer concentration (Figure 5.9) due to the increased precipitation of hydroxyapatite. To account for reduction in Ca$^{2+}$ concentration on Ag NP aggregation kinetics with the increase in buffer concentration, the attachment efficiency curves are presented as a function of Ca$^{2+}$ remaining in solution in Figure 3c. In general, the attachment efficiency curves as function of counter-ion concentration show two aggregation regimes defined as
reaction limited aggregation (RLA) and diffusion limited aggregation (DLA; Figure 5.8a-c). Figure 5.8a-c shows that the attachment efficiency curves shift to the left (i.e. to lower counter-ion concentrations) with the increased buffer concentration, suggesting that phosphate buffer enhances the aggregation of cit-Ag NPs. At the same counter-ion concentration under RLA regime, the attachment efficiency increases with the increase in buffer concentration (Figure 5.8a-c). This behavior can be attributed to 1) added Na$^+$ and K$^+$ used to buffer and adjust the solution pH, 2) sequestration of calcium ions by phosphate anions, and 3) reduction in Ag NP surface charge due to sorption of phosphate anions on the surface of Ag NPs.

The final concentration of K$^+$ added due to buffer solution and Na$^+$ added due to pH adjustment by NaOH are < 1 and 0.4 mM, respectively for all solutions used in this study. These concentrations of K$^+$ and Na$^+$ are well below cit-Ag NP aggregation threshold (ca. > 40 mM Na$^+$, Figure 5.8a) and are much less than the shift in the attachment efficiency curves (ca. 100 mM shift for 0.05 and 0.25 mM phosphate buffer concentrations, Figure 5.8a). Therefore, the small concentrations of K$^+$ and Na$^+$ added from phosphate buffer and NaOH solutions cannot explain the differences in Ag NP aggregation kinetics. Similarly, sequestration of Ca$^{2+}$ ions is higher at higher buffer concentrations (Figure 5.9), resulting in more pronounced shift in the attachment efficiency curves when expressed as a function of Ca$^{2+}$ remaining in solution (Figure 5.8c) compared to Ca(NO$_3$)$_2$ (Figure 5.8b). Thus, calcium sequestration by phosphate anions does not explain the differences in Ag NP aggregation behavior. Consequently, the differences in Ag NP aggregation behavior in the presence of phosphate buffer solution can be attributed to the higher adsorption of phosphate anions on the surface of cit-Ag NPs at higher buffer concentrations, leading to
a decrease in the surface charge (indicated by decrease in zeta potential) of cit-Ag NPs at higher buffer concentrations (Figure 5.10). Due to the low concentration of cit-Ag NPs used in this study (270.25 μg L\(^{-1}\)), it was not possible to measure the zeta potential of Ag NPs in the presence of buffer using laser Doppler electrophoresis. Therefore, to demonstrate the impact of buffer concentration on the surface charge of cit-Ag NPs, we measured the zeta potential of cit-Ag NPs at higher buffer concentrations (0.5-2.5 mM) and higher cit-Ag NP concentration (5.4 mg L\(^{-1}\)). Cit-Ag NPs are negatively charged without any buffer (zeta potential of - 41.7±2.0 mV). The addition of phosphate anions results in a decrease in the zeta potential of cit-Ag NPs, which can be attributed to the sorption/complexation of the phosphate anions on Ag NP surfaces\(^{172,173}\) due to the high affinity of phosphate anions to silver (\(K_{sp} = 8.89 \times 10^{-17}\) M)\(^{174}\).

The zeta potential of cit-Ag NPs decreases rapidly at low buffer concentrations (ca. from -41.7±2.0 mV to -30.4±0.7 mV for an increase in buffer concentration from 0 to 1 mM), followed by a slower decrease at higher buffer concentrations (ca. 1-2.5 mM; Figure 5.10). The zeta potential of Ag NPs tends to a stable value of ca. -29±1.0 mV at higher buffer concentrations (calculated as the average and standard deviation of the zeta potential measured at buffer concentration in the range of 1.0-2.5 mM). This is likely due to the saturation of the surface of Ag NPs with phosphate anions at higher buffer concentrations\(^ {175}\).

The CCC as a function of phosphate buffer concentration decreases with the increase in buffer concentration (Figure 5.11a and 5.11b), which can be attributed to the reduction in the zeta potential of Ag NPs due to the sorption of phosphate anions on the surface of Ag NPs. The CCC decreases rapidly at low buffer concentrations and tends to a
stable value with the increase in buffer concentration (ca. 90.9 mM NaNO₃, Figure 5.11a, 0.10 mM intial Ca(NO₃)₂, Figure 5.11b, 0.017 mM free Ca²⁺, Figure 5.11c). This behavior is in good agreement with the zeta potential reduction due to the sorption of phosphate anions on the surface of Ag NPs (Figure 5.10). Therefore, the reduction in the CCC with the increase in buffer concentration can be attributed to the decrease in zeta potential. The tendency in CCC to a stable value at high buffer concentrations can be attributed to the saturation of Ag NPs surface with phosphate anions at higher buffer concentrations. The decrease in the CCC for citrate Ag NPs with the increase in phosphate buffer concentration is in good agreement with the reported decrease in the CCC for charge stabilized-Ag NPs with the increase in carbonate buffer concentration²⁹,³⁰. For instance, the CCC of Na⁺ for charge stabilized-Ag NPs decreases from approximately 150 mM Na⁺ in the absence of buffer to approximately 25 mM Na⁺ at higher carbonate buffer concentrations (ca. >0.05 mM NaHCO₃)²⁹.

Herein, cit-Ag NPs aggregation can be explained by both chemical and physical interactions. Phosphate anions can replace the citrate coating and bind to the NP surface through its oxygen atoms, resulting in phosphate coated Ag NPs.¹⁷² Aggregation of phosphate coated Ag NPs takes place through physical surface charge screening by counter-ions (Na⁺ and Ca²⁺) according to DLVO theory.⁴⁷

5.3.2. Aggregation impacted by fulvic acid

The UV-Vis spectra of cit-Ag NPs 10 minutes after interaction with phosphate buffer in the presence of 1 mgL⁻¹ SRFA show a decrease in the UV-Vis absorbance at λ = 396 nm concurrent with a broadening of the peak with NaNO₃, and formation of a second peak with Ca(NO₃)₂, which can be attributed to the aggregation of primary cit-Ag NPs
(Figure 5.12a-d). At the same NaNO₃ and Ca(NO₃)₂ concentrations, primary NP peak at 396 nm decays to higher absorbance and peak shoulder is narrower in the presence of SRFA compared to those in the absence of SRFA (Figure 5.9a and b). For example, cit-Ag NP peak at 396 nm in 1 mM buffer and 100 mM NaNO₃ decays to higher absorbance (~0.24, Figure 5.9a) in the presence of SRFA compared to the absence of SRFA (~0.08, Figure 5.9a) and the peak is broader in the absence of SRFA suggesting that SRFA increase the stability of cit-Ag NPs against cation induced aggregation. Similarly, cit-Ag NP peak at 396 nm in 1 mM buffer and 0.25 mM Ca(NO₃)₂ mM decays to higher absorbance (~0.30, Figure 5.9b) in the presence of SRFA compared to the absence of SRFA (~0.15, Figure 5.9b) and the peak is broader in the absence of SRFA suggesting that SRFA increase the stability of cit-Ag NPs against cation induced aggregation. However, SRFA does not entirely eliminate the impact of phosphate anions on the aggregation of cit-Ag NPs.

The attachment efficiency of cit-Ag NPs in the presence of SRFA and at different phosphate buffer concentrations is presented in Figure 5.13a and b for NaNO₃ and Ca(NO₃)₂. Within the RLA regime, at the same electrolyte concentration, the attachment efficiency increase with the increase in buffer concentration, suggesting that the increased buffer concentration reduced the stability of cit-Ag NPs, presumably due to the sorption of phosphate anions on the surface of cit-Ag NPs in the presence of SRFA. The CCC shifted to lower counter-ion concentrations (157.7±4.4 mM NaNO₃ and 1.5±0.03 Ca(NO₃)₂) at higher buffer concentration compared to the CCC (265.3±2.5 mM NaNO₃ and 1.8±0.21 mM Ca(NO₃)₂) at lower buffer concentration (Figure 5.13a and b, Table 5.1). It is worth noting that in the case of Ca²⁺ counter-ions (Figure 5.13b), the attachment efficiency does not show a clear separation between the RLA and DLA regimes, and the attachment
efficiency reaches values > 1.0 at high Ca\textsuperscript{2+} concentration. This behavior can be attributed to inter-particle bridging by humic acid aggregates as observed elsewhere.\textsuperscript{43} Enhanced aggregation has been attributed to inter-particle bridging of NPs by humic acid aggregates which form through the complex formation between humic acid macromolecules with Ca\textsuperscript{2+} ions.\textsuperscript{176}

Furthermore, at the same concentration of buffer, the CCC shifted to higher electrolyte concentration in the presence of SRFA (Figure 5.13a and b) compared to the CCC values in the absence of SRFA (Figure 5.8 and Table 5.1). It has been demonstrated that fulvic acid replaces citrate coating and form a surface coating on Ag NPs and increase their stability.\textsuperscript{109} Several studies have demonstrated the shift in CCC to higher counter-ion concentrations in the presence of SRFA.\textsuperscript{1,247,162} However, SRFA did not completely inhibit surface charge screening and NP aggregation by NaNO\textsubscript{3} and Ca(NO\textsubscript{3})\textsubscript{2} electrolytes.\textsuperscript{47,111} Additionally, SRFA did not prevent the reduction in Ag NP stability due to their interaction with phosphate anions.

5.3.3. Environmental Implications and Conclusions

Buffers have been widely used in environmental and toxicological studies to maintain constant pH values without investigating the impact of the buffer on the stability of the NPs. Here, we systematically investigated the impact of phosphate buffer concentration (as an example) and SRFA on the aggregation kinetics of cit-Ag NPs by NaNO\textsubscript{3} and Ca(NO\textsubscript{3})\textsubscript{2} by measuring the aggregation kinetics of cit-Ag NPs at different phosphate buffer concentrations (ca. 0 to 1.0 mM). The increase in buffer resulted in a decrease in the CCC both in the presence and absence of SRFA. The CCC tends to a constant value at ca. 1.0 mM phosphate buffer concentration for the concentrations of cit-
Ag NPs used in this study (270.25 µg L\(^{-1}\)). The buffer concentration required to reach a stable CCC depends on the concentration of Ag NPs, and decrease with the decrease in NP concentrations as lower buffer anions concentration will be required to fully coat the surface of Ag NPs.

The majority of NP aggregation kinetic studies has focused on the role of counterions (ca. Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\))\(^{43,70,177}\), and few studies investigated the impact of anions such as carbonates and phosphates on NP aggregation.\(^{178,47,179}\) Nonetheless, buffers (e.g. carbonate, phosphate, MOPS, etc.) are widely used in aggregation kinetic studies to maintain a constant pH.\(^{56}\) These studies reported different CCC values. For instance, El Badawy et al. 2012\(^{48}\) reported higher CCC (ca. 70 mM NaNO\(_3\) and 5 mM Ca(NO\(_3\))_2) for 58 nm cit-Ag NPs compared to the study by Li 2011 (70 mM NaNO\(_3\) and 5 mM Ca(NO\(_3\))_2) for 64 nm cit-Ag NPs\(^{67}\). Based on our findings, the lower CCC values reported in Li et al. (2011) compared to those reported by Badawy et al. 2012\(^{48}\) can be attributed (at least partially) to the higher carbonate buffer concentration used in the former study.

Considering the lower expected environmental concentrations of Ag NPs (0.01-10 µg L\(^{-1}\))\(^{145,146}\) compared to those investigated in this study (270.25 µg L\(^{-1}\)), it is likely that at lower NP concentrations, the CCC will tend to a constant value at lower phosphate anion concentrations as lower phosphate anion concentrations will be required to saturate the surface of Ag NPs. Considering the higher NP concentrations typically studied in the literature e.g. (mg L\(^{-1}\)), it is likely that studies performed at high NP concentration underestimates the attachment efficiencies and CCC, and these values need to be considered carefully.
Table 5.1. CCC values in the presence and absence of SRFA

<table>
<thead>
<tr>
<th>Buffer concentration (mM)</th>
<th>Electrolyte</th>
<th>CCC in the presence of SRFA (mM)</th>
<th>CCC in the absence of SRFA (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>NaNO₃</td>
<td>265.3 ± 2.5</td>
<td>232.0 ± 1.4</td>
</tr>
<tr>
<td>1.00</td>
<td>NaNO₃</td>
<td>157.7 ± 4.4</td>
<td>90.9 ± 2.2</td>
</tr>
<tr>
<td>0.05</td>
<td>Ca(NO₃)₂</td>
<td>1.8 ± 0.21</td>
<td>0.7 ± 0.001</td>
</tr>
<tr>
<td>1.00</td>
<td>Ca(NO₃)₂</td>
<td>1.5 ± 0.03</td>
<td>0.1 ± 0.008</td>
</tr>
</tbody>
</table>
Figure 5.1. UV–Vis spectra of cit-Ag NPs (270.25 μg L\(^{-1}\)) collected 10 min after mixing with different concentrations of NaNO₃ (a and b) and Ca(NO₃)\(_2\) (c and d) in different concentrations of potassium phosphate monobasic buffer (pH 7.0): (a and c) 0.05 mM, and (b and d) 1 mM. Figure legend represents the electrolyte concentrations.
Figure 5.2. UV-Vis spectra of cit-Ag NPs (270 µg L\(^{-1}\)) collected 10 min after mixing cit-Ag NPs with NaNO\(_3\) in different concentrations of buffer: (a) 0 mM, (b) 0.05 mM, (c) 0.25 mM, (d) 0.5 mM, and (e) 1 mM. Figure legend represents the electrolyte concentrations.
Figure 5.3. UV-Vis spectra of cit-Ag NPs (270 µg L⁻¹) collected 10 min after mixing Ag NPs with (a) 100 mM NaNO₃ and (b) 0.25 mM Ca(NO₃)₂ at different concentrations of buffer (0-1 mM). All analysis were performed at pH 7.0. Figure legend represents potassium phosphate monobasic buffer concentrations.
Figure 5.4. UV-Vis spectra of cit-Ag NPs (270 µg L\(^{-1}\)) collected 10 min after mixing Ag NPs with Ca(NO\(_3\))\(_2\) in different concentrations of buffer: (a) 0 mM, (b) 0.05 mM, (c) 0.25 mM, (d) 0.5 mM, and (e) 1 mM. Figure legend represents the electrolyte concentrations.
Figure 5.5. Evolution of the UV–Vis absorbance at \( \lambda_{396} \) of cit-Ag NPs (270.25 \( \mu \)g L\(^{-1} \)) as a function of time immediately after mixing with different concentrations of NaNO\(_3\) (a and b) and Ca(NO\(_3\))\(_2\) (c and d) in different concentrations of buffer (pH 7.0): (a and c) 0.05 mM, and (b and d) 1 mM.
Figure 5.6. UV-Vis absorbance at 396 nm of cit-Ag NPs (270 µg L⁻¹) mixed with different concentrations of NaNO₃ at different concentrations of buffer: (a) 0 mM, (b) 0.05 mM, (c) 0.25 mM, (d) 0.5 mM, and (e) 1 mM. Figure legends represent NaNO₃ concentrations.
Figure 5.7. UV-Vis absorbance at 396 nm of Ag NP mixed with different concentrations of Ca(NO₃)₂ at different concentrations of buffer: (a) 0 mM, (b) 0.05 mM, (c) 0.25 mM, (d) 0.5 mM, and (e) 1 mM. Figure legends represent Ca(NO₃)₂ concentrations.
Figure 5.8. Attachment efficiency of cit-Ag NPs (270.25 μg L⁻¹) at different concentrations of phosphate buffer reacted with: (a) NaNO₃, (b) Ca(NO₃)₂, and (c) Ca²⁺ remaining in solution. All experiments were performed at pH 7.0. Figure legend represents potassium phosphate monobasic buffer concentration.
Figure 5.9. Concentration of Ca$^{2+}$ as a function of initial calcium concentration at different buffer concentration. The reduction of Ca$^{2+}$ is due to precipitation of hydroxyapatite.
Figure 5.10. Zeta potential of cit-Ag NPs as a function of potassium phosphate monobasic buffer concentration. Measurements were performed at higher NP concentration (5.4 mg L$^{-1}$). Dotted line is used to guide the reader eye.
Figure 5.11. Critical coagulation concentration of cit-Ag NPs (270.25 μg L\(^{-1}\)) as a function of phosphate buffer concentration in the presence of monovalent and divalent electrolytes (a) NaNO\(_3\), (b) Ca(NO\(_3\))\(_2\) and (c) Ca\(^{2+}\) remaining in solution following precipitation of hydroxyapatite. Dotted line is used to guide the reader eye.
Figure 5.12. UV–vis spectra of cit-Ag NPs (270.25 μg L\(^{-1}\)) mixed with 1 mg L\(^{-1}\) Suwannee River fulvic acid (SRFA) collected 10 min after mixing with different concentrations of NaNO\(_3\) (a and b) and Ca(NO\(_3\))\(_2\) (c and d) in different concentrations of buffer (pH 7.0): (a and c) 0.05 mM, and (b and d) 1 mM. Figure legend represents the electrolyte concentrations.
Figure 5.13. Attachment efficiency of cit-Ag NPs (270.25 μg L\(^{-1}\)) at different concentrations of phosphate buffer reacted with: (a) NaNO\(_3\), (b) Ca(NO\(_3\))\(_2\) in the presence of SRFA (1 mg L\(^{-1}\))
CHAPTER 6

CONCLUSIONS
The overall aim of this PhD was to investigate the effects of Ag NPs physicochemical properties (e.g., size, surface charge, surface coating, etc.) and water chemistry (e.g., buffer concentration, organic ligands, NOM) in controlling Ag NPs colloidal stability. This goal was achieved by systematically 1) review and rationalize the many studies that investigated the aggregation kinetics of Ag NPs (conclusion 1-5); and 2) investigate the aggregation kinetics of Ag NPs under specific conditions that has not been studied in the literature, in particular buffer concentration and organic ligands with different structures (conclusion 6-11).

Ag NP stability was determined by measuring Ag NP aggregation kinetics in different media – such as carbonate and phosphate buffer, organic ligands of different chemical structures, and counter-ions by determining NP aggregation rate. NP aggregation rate was determined by measuring the growth in Ag NP aggregate size as a function of time using dynamic light scattering, or the loss of the primary Ag NP as a function of time using UV-Vis spectroscopy, which were then used to determine NP attachment efficiency and the CCC. The CCC provides a measure of NP stability under the specific experimental conditions. The overall conclusions of these studies are summarized below:

1- The CCC is independent of NP concentration for pure electrostatic interactions. However, in the presence of chemical constituents of high affinity to NPs such as cystine, carbonate and phosphate anions, the CCC is NP concentration dependent.

2- The CCC for Ag NPs increases with the decrease in particle size at a fixed zeta potential for monovalent electrolytes. Despite the strongly negative association between the CCC and particle size for monovalent electrolytes, no clear trend was observed between CCC and Ag NP size for divalent electrolytes.
3- The CCC for Ag NPs decreases with light irradiation due to the degradation of surface coating and reduction of the surface charge magnitude. Given that the majority of Ag NP aggregation kinetics have been performed under laboratory condition, where the majority of the radiation is filtered, this finding may imply that in environmental systems Ag NPs will be less stable than those reported in laboratory-based studies.

4- The majority of NP aggregation kinetic studies has focused on the role of counter-ions (ca. Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\)) and few studies investigated the impact of anions such as carbonates and phosphates on NP aggregation. Nonetheless, buffers (e.g. carbonate, phosphate and MOPS) are widely used in aggregation kinetic studies to maintain a constant pH.\(^{56}\) These studies reported different CCC values. For instance, El Badawy et al. reported higher CCC for 58 nm cit-Ag NPs compared to the study by Li et al. for 64 nm cit-Ag NPs.\(^{48, 67}\) Based on our findings, this difference in CCC value can be attributed to the higher carbonate buffer concentration used in the former study. Considering the lower concentrations of Ag NPs expected in the environment (0.01–10 \(\mu\)g L\(^{-1}\))\(^{145, 146}\) compared to those investigated in our study (270.25 \(\mu\)g L\(^{-1}\)), it is likely that at lower NP concentrations, the CCC will tend to a constant value at lower phosphate anion concentrations as a lower phosphate anion concentration is required for saturating the Ag NP surface. Considering the higher NP concentrations typically studied in the literature e.g. mg L\(^{-1}\), it is likely that studies performed at high NP concentration underestimates the attachment efficiencies and CCC, and as a result a careful consideration of these values is suggested.

5- Fully coated, sterically stabilized Ag NPs do not aggregate even at high ionic strength. However, the stability or CCC of partially coated Ag NPs decreases with the decrease
in surface coverage by the capping agent. Also, sterically stabilized Ag NPs can be destabilized by surface coating replacement due to their higher affinity to Ag NPs. Therefore, sterically stabilized NPs may become unstable in the presence of natural organic molecules (e.g., cysteine) of higher affinity to the NP surfaces compared to those used to sterically stabilize NPs (e.g., PVP).

6- Buffers have been widely used in environmental and toxicological studies to maintain constant pH values without investigating the impact of the buffer on the stability of Ag NPs. The CCC for Ag NPs decreased with the increase in phosphate buffer concentration in the presence and absence of SRFA. The CCC tends to a constant value at ca. 1.0 mM phosphate buffer concentration for the concentration of cit-Ag NPs used in this study (270.25 μg L\(^{-1}\)). The required buffer concentration for a stable CCC depends on the concentration of Ag NPs, and decrease with the decrease in NP concentration as lower buffer anions concentration will be required to fully coat the surface of Ag NPs.

7- Cystine significantly impacts the stability of citrate, PVP and PEG coated Ag NPs, resulting in a concentration dependent aggregation of Ag NPs, with a shift in the CCC toward lower concentrations of cystine at lower concentration of Ag NPs. SRFA reduces the cystine-induced aggregation of Ag NPs within the limit of cystine solubility in water, likely due to the steric effect of SRFA. Thus, in natural surface waters, cystine can play an important role in determining the fate and behavior of cit-Ag NPs, and further research is required to investigate the interplay between cystine, electrolytes and NOM on the stability of Ag NPs. Due to the higher affinity of the cysteine thiol
groups to Ag⁺ and Ag surfaces compared to the cystine disulfide group, a similar concentration dependent aggregation of Ag NPs in the presence of cysteine is expected.

8- Thiol containing biomolecules play an important role in determining Ag NPs aggregation behavior. In particular, the molecular structure (functional groups) of the organic ligands play a significant role in determining the stability of NPs. L-cys decreases the stability of Ag NPs, whereas NAL-cys increases the stability of Ag NPs. Additionally, whereas SRFA increased the stability of Ag NPs, addition of a mixture of SRFA and L-cys decreased the stability of Ag NPs. It is worth noting here that both L-cys and NAL-cys contain the same number of N and S atoms per molecule. However, NAL-cys is a derivative of cysteine wherein an acetyl group is attached to the nitrogen atom, thus neutralizing the positive charge on the amine group in the L-cys molecule.

This study illustrates the need for a better and more in-depth characterization of NOM at the molecular level to better understand the role of NOM in determining the environmental behavior and effects of NPs. A combination of analytical bulk and molecular level characterization tools such as NMR, FTIR, and ultrahigh resolution-mass spectroscopy techniques such as Fourier transform-ion cyclotron-mass spectroscopy are likely needed to achieve such in depth understanding of NOM and its role in modulating NP environmental fate and behavior.

9- Both L-cys and NAL-cys have been used in ecotoxicological studies to scavenge dissolved Ag ions. However, no study has considered the cysteine-induced Ag NP destabilization on Ag NP uptake and toxicity. The decreased stability of Ag NPs in the presence of L-cys could contribute to the decreased uptake and/or toxicity of Ag NPs reported in the literature, which should be further investigated in future studies. Given
that both L-cys and NAL-cys equally reduce the dissolution of Ag NPs and that NAL-cys increases the stability of Ag NPs in electrolyte solution, NAL-cys is recommended as a better alternative in ecotoxicological studies to scavenge dissolved Ag ions.

10- Whereas the concentration of SRFA does not significantly impact the stability of Ag NPs, the increased concentration of YRNOM increases the stability of Ag NPs due to the increased selective sorption of higher molecular weight NOMs on the surface of Ag NPs, which increases the steric stabilization of Ag NPs. SRFA, extracted by XAD resin, represents a subgroup of NOM characterized by low molecular weight; whereas YRNOM, extracted by ultrafiltration and thus containing a mixture of fulvic and humic acids as well as other NOM molecules that are more complex and contain molecular formulas of higher molecular weight, which selectively sorb on the surface of NPs.63 Therefore, this study illustrates that the impact of NOM on NP stability may also depend on the extraction protocol.

11- Studying both XAD-extracted fractions together with total NOM fractions “isolated by ultrafiltration” is ideal to better understand the roles of NOM in determining NP environmental and biological interactions. NOM isolates are simpler and therefore are better suited for mechanistic understanding of NOM-NP interactions, whereas total NOM samples are more environmentally realistic and can provide better more insight into the expected environmental behaviors of NPs. Finally, this work underscores the importance of understanding the impact of NOM molecular properties (e.g. molecular composition and structure, molecular weight) on NP stability in both ecotoxicological media as well as in the natural environment, and thus the fate and effects of NPs.
Outlook

The overall outcome of this results suggest that the stability of NPs is complex and cannot be described by an individual parameter such as ionic strength, or concentration of NOM, that has been the focus of many studies in the literature. Rather, NP stability should be determined by a combination of NP and medium physicochemical properties.

The physicochemical properties of NPs such as particle size and surface coating play important roles in determining Ag NP stability as discussed above. However other NP properties such as chemical composition, shape and dispersity might have significant impact on NP stability, which has not received much attention in the literature and are worth investigating in future studies.

In addition to counter-ions that have been the focus of the majority of NP aggregation studies in the literature, specific sorption of water constituents such as buffer, anions, organic ligands with high affinity to NP surface (e.g., cysteine to Ag NPs) play an equally important role in controlling NP stability, especially at environmentally relevant NP concentrations (e.g., ng to µg L$^{-1}$ range). Therefore, focusing only on the physical interactions (e.g. attraction and repulsion) of NPs when taking into account water chemistry in modeling environmental fate and behavior of NPs might lead to erroneous conclusions. Overall, these findings are important to underpin nanomaterial risk assessment and environmental fate and behavior studies.

Considering that NP concentration in our study is greater than those typically predicted to be in the environment (ng to µg L$^{-1}$), future studies should attempt to investigate NP stability at lower, more environmentally relevant NPs concentrations as the probability of NP-NP interactions might become negligible at such low NP concentrations.
Single particle ICP-MS (SP-ICP-MS) is one of the very few, perhaps only, method to study NP aggregation in this concentration range.

Considering the wide range of NOM concentrations in the natural environment, future studies should investigate the effect of NOM on NP behaviors at different NOM to Ag NP concentration ratios that are more environmentally relevant. The significant differences in the impact of YRNOM and SRFA concentrations on Ag NP stability suggests the need to further understand 1) the effect of NOM isolates and more environmentally relevant total NOM samples on the behaviors of Ag NPs 2) The effect of selective sorption of NOM molecules on the surface of Ag NPs on their environmental behaviors.

Finally, this PhD focused on mechanistic understanding of effect of NP and media properties on NP stability and therefore, all experiments were performed in relatively simple media. Future studies is suggested using an optimization method e.g. response surface methodology to investigate the interplay between the different factors and how they may affect NP environmental behaviors.
REFERENCES


16. Merrifield, R. C.; Stephan, C.; Lead, J., Determining the Concentration Dependent Transformations of Ag Nanoparticles in Complex Media: Using SP-ICP-MS and Au@ Ag Core–Shell Nanoparticles as Tracers. *Environmental Science & Technology* **2017**.


59. Filella, M., Colloidal properties of submicron particles in natural waters. IUPAC series on analytical and physical chemistry of environmental systems 2007, 10, 17.
71. Lodeiro, P.; Achterberg, E. P.; Pampin, J.; Affatati, A.; El-Shahawi, M. S., Silver nanoparticles coated with natural polysaccharides as models to study AgNP aggregation kinetics using UV-Visible spectrophotometry upon discharge in complex environments. Science of the Total Environment 2016, 539, 7-16.
86. Pokhrel, L. R.; Dubey, B.; Scheuerman, P. R., Impacts of select organic ligands on the colloidal stability, dissolution dynamics, and toxicity of silver nanoparticles. *Environmental science & technology* 2013, 47, (22), 12877-12885.


120. Foldbjerg, R.; Dang, D. A.; Autrup, H., Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Archives of toxicology 2011, 85, (7), 743-750.


170. White, P. J.; Hammond, J. P., The Sources of Phosphorus in the Waters of Great Britain All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. *Journal of Environmental Quality* **2009**, *38*.
175. Devi, L. B.; Mandal, A. B., Interaction of Silver Nanoparticles with DNA Building Blocks. **2011**.
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APPENDIX D: LICENSE AGREEMENT FOR CHAPTER 3

Title: The concentration-dependent aggregation of Ag NPs induced by cysteine
Author: K. Afshar, S. Gibson, R. Harris, M. R. Baabusha
Publication: Science of The Total Environment
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APPENDIX E: LICENSE AGREEMENT FOR CHAPTER 4

Title: Potential impact of natural organic ligands on the colloidal stability of silver nanoparticles

Author: Kamelia Alshinnia, Brandon Narrone, Mohammad Raajeeborah

Publication: Science of The Total Environment

Publisher: Elsevier

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