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 (α) 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 α 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-AgNPs) 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-AgNPs to lower counter ion concentration and an increase of α at the same counter ion concentration, both in the absence and presence of SRFA. SRFA stabilizes AgNPs and increases the CCC to higher counter ion concentrations.

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

1. Introduction

Silver nanoparticles (AgNPs) are nowadays used in a large number of commercial products due to their antimicrobial properties¹, resulting in release of AgNPs to the environment^{2, 3}, with estimated surface water concentrations in the ng to $\mu\text{g L}^{-1}$ range.^{4, 5} Once in the environment, AgNPs undergo several transformations such as dissolution⁶, aggregation⁷, sulfidation⁸, photochemical, and biological transformations.⁹⁻¹¹ These transformations of AgNPs can impact their fate and effects in the environment.⁹⁻¹¹ Nanoparticle (NP) aggregation is one of the key processes determining their reactivity¹²⁻¹⁴, bioavailability^{15, 16}, toxicity¹⁷⁻²⁰ and environmental fate and effects.¹⁶

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).^{7, 21, 22} NP suspension stability is typically characterized by two fundamental parameters (attachment efficiency, α ; 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).^{23, 24} The Critical coagulation concentration (CCC) is the minimum concentration of counter ions required to completely destabilize the NP suspension. The attachment efficiency (α) has been recently a focus of discussion due to its importance to construct NP fate models.^{16, 25} Thus, the recent spike in publications of empirically determined α 's or related parameters (*e.g.* Debye length) for use in such models.^{7, 23, 26-30} Nonetheless, there is still a need to better understand the factors determining α , among which NP and medium physicochemical properties^{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.^{12, 28, 31-36}

For instance, Stemig *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.¹² Several studies have investigated the aggregation kinetics of AgNPs; some studies used buffer such as carbonate buffers to

maintain a stable pH^{26, 27, 37}, while others did not use any buffer to prevent interferences due to the presence of the buffer.^{7, 38} These studies have reported significant variability in the measured α and CCC values for citrate-AgNPs, which were attributed to differences in experimental conditions such as media composition (*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.^{38, 39} However, none of these studies have systematically investigated the effect of buffer type and concentration on the aggregation behavior of AgNPs.

Therefore, the aim of this study is to assess the effect of phosphate buffer on the stability of citrate-AgNPs. The stability of AgNPs was quantified by measuring AgNP aggregation kinetics using UV-vis and determining the α 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 AgNPs concentration (*e.g.* 270.25 $\mu\text{g L}^{-1}$). The concentration of AgNPs used in this study is less than those typically used in aggregation kinetic studies in the literature.³⁸ However, it is still higher than those estimated to occur in environmental systems based on exposure modeling approaches.^{4, 40} Phosphate buffer was used as an environmentally and biologically relevant buffer in this study.⁴¹ Phosphate is commonly found in environmental⁴² and biological and toxicological media.⁴³⁻⁴⁵ 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.^{46, 47} 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 AgNP aggregation kinetics, and hence, the environmental fate, behavior and impact of AgNPs.

2. Methodology

Synthesis and characterization of silver nanoparticles

Citrate-coated silver nanoparticles (cit-AgNPs) were synthesized and characterized using a previously reported method.⁴⁸ 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 M Ω .cm) and were then kept at 4°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 AgNP suspension was then stored in dark overnight and cooled to 4°C. 200 ml of AgNP suspension was cleaned by pressurized stirred ultrafiltration cell (Amicon, 1 kDa regenerated cellulose membrane, Millipore) to remove the excess reagents before use. AgNP 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 AgNPs 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 AgNPs 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-AgNPs 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-AgNPs (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-AgNPs was collected by a UV-vis spectrophotometer. All spectra were collected using a 100 mm path length cuvette, which allows analysis of AgNPs at relatively low concentration (270.25 μ g L⁻¹).⁷ The UV-vis spectrum of

AgNP 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 (Fig. S1).

Solution Chemistry

The effect of phosphate buffer on the aggregation kinetics of cit-AgNPs 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 $\text{Ca}(\text{NO}_3)_2$ (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 1 mM , respectively. Therefore, any differences in AgNPs aggregation kinetics, is due to the variation in phosphate buffer concentration (see detailed discussion below). 1 M NaNO_3 and 3 mM $\text{Ca}(\text{NO}_3)_2$ electrolyte solutions were prepared in ultrahigh purity water or diluted buffer. Aliquots ($250 \text{ }\mu\text{l}$) of AgNPs 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 $\text{Ca}(\text{NO}_3)_2$) to achieve a final volume of 10 mL for UV-vis experiment. These dilutions resulted in a final concentration of $270.25 \text{ }\mu\text{g L}^{-1}$ AgNPs, and different concentration of electrolytes (40-260 mM NaNO_3 and 0.045-1 mM $\text{Ca}(\text{NO}_3)_2$) 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 AgNP 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^{1-} , K^+ , PO_4) 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 AgNP suspensions at the same concentrations (*e.g.* 270.25 $\mu\text{g L}^{-1}$) 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 AgNPs and 3.2 mL of ultrahigh purity water giving a mixture of 5.4 mg L^{-1} AgNPs 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 AgNPs 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 $\mu\text{g L}^{-1}$ AgNPs, 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-AgNPs was investigated by measuring the zeta potential of AgNPs (5.4 mg L^{-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 AgNP 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} AgNPs, and different concentrations of phosphate buffer (0-2.5 mM).

Aggregation kinetics

AgNP 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 ($\lambda = 396 \text{ nm}$) of individual AgNPs.⁴⁹ The slope of the loss in the UV-vis absorbance at $\lambda = 396 \text{ nm}$ within the first 10 s after mixing of AgNPs 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 AgNP

aggregation only as demonstrated in previous studies.^{7, 50} The attachment efficiency (α) or the inverse stability ratio (W) were calculated according to Eq. 1.

$$\alpha = \frac{1}{W} = \frac{K_{slow}}{K_{fast}} \quad (\text{Eq.1})$$

Where K_{slow} and K_{fast} are the aggregation rate constant under reaction (RLA) and diffusion (DLA) limited aggregation regimes. K_{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.

3. Results and Discussion

3.1. Impact of phosphate buffer on the aggregation behavior of cit-AgNPs

The UV-vis spectra of cit-AgNPs, 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 Fig. 1a and b, respectively (full data set at 0, 0.05, 0.35, 0.5 and 1 mM phosphate buffer concentrations is presented in Fig. S2, a-e). At low buffer concentration (0.05 mM, Fig. 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-AgNPs (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-AgNPs indicates the loss of primary cit-AgNPs by aggregation in the presence of NaNO_3 as observed elsewhere.⁷ It is well-known that cit-AgNPs are electrostatically stabilized, and that the increase in counter ion concentration (here Na^+) results in surface charge screening, allowing AgNPs to stick together and aggregate.

At high buffer concentrations (1 mM, Fig. 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 (Fig 1a), potentially due to the formation of larger AgNP aggregates that absorb and scatter light at higher wavelengths.⁵¹ Furthermore, higher concentration of NaNO₃ (*ca.* 100-260 mM, Fig. 1a) was required to induce aggregation of cit-AgNPs at lower buffer concentration (0.05 mM) compared to those (40-100 mM NaNO₃, Fig. 1b) required to induce aggregation at higher buffer concentration. In general, the increase in buffer concentration in 0-1 mM range (Fig. S2, a-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 AgNPs with the increase in buffer concentration.

At the same concentration of NaNO₃ (*ca.* 100 mM, Fig. S4a), 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-AgNPs at higher buffer concentration than at lower buffer concentration. The increased cit-AgNPs aggregation with the increased phosphate buffer concentration is presumably due to the sorption of phosphate anions on the surface of AgNPs⁵², resulting in increased reduction of AgNP surface charge at higher buffer concentrations (see discussion below).

The UV-vis spectra of cit-AgNPs after 10 min of mixing with Ca(NO₃)₂ in different concentration of phosphate buffer (0.05 and 1.0 mM) are presented in Fig. 1c and d, respectively (full data set at 0, 0.05, 0.35, 0.5 and 1 mM phosphate buffer concentrations is presented in Fig. S3 a-e). Similarly to NaNO₃, the increase in Ca(NO₃)₂ results in increased loss of the UV-vis absorbance at 396 nm, broadening of the absorbance peak of the as-synthesized cit-AgNPs, and the formation of a second peak centered on *ca.* 550 nm, indicating the increased aggregation of cit-AgNPs with the increase in Ca(NO₃)₂ concentration. Furthermore, higher concentration of Ca(NO₃)₂ (*ca.* 0.25-0.8 mM, Fig. 1c) was required to induce aggregation of cit-AgNPs at lower buffer concentration (0.05 mM) compared to higher buffer concentration (0.045-0.25 mM Ca(NO₃)₂, Fig. 1d). In general, the increase in buffer concentration in 0-1 mM range (Fig. S3 a-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 AgNPs with the increase in buffer concentration.

At the same concentration of $\text{Ca}(\text{NO}_3)_2$ (*ca.* 0.25 mM, Fig. S4b), 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 AgNPs by increased aggregation at higher buffer concentrations. In other words, $\text{Ca}(\text{NO}_3)_2$ is more efficient in aggregating cit-AgNPs at higher buffer concentration (Fig. S4b) than at lower buffer concentration.

To measure AgNP aggregation kinetics' parameters (e.g. α and CCC), the loss of primary particles due to aggregation in the presence of NaNO_3 and $\text{Ca}(\text{NO}_3)_2$ at different buffer concentrations was monitored by measuring the UV-vis absorbance at $\lambda = 396$ (Fig. 2, full data set of all different buffer concentrations is presented in Fig. S5 and S6). 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 (Fig. 2a-d). In general, lower electrolyte concentrations (*ca.* 40-120 NaNO_3 , Fig. 2b and 0.045-0.25 mM $\text{Ca}(\text{NO}_3)_2$, Fig. 2d) were required to induce aggregation of cit-AgNPs at higher buffer concentration compared to lower buffer concentration (*ca.* 50-260 NaNO_3 , Fig. 2a and 0.25-0.9 mM $\text{Ca}(\text{NO}_3)_2$, Fig. 2c).

The attachment efficiencies of cit-AgNPs as a function of initial electrolyte concentration (NaNO_3 and $(\text{CaNO}_3)_2$) in the presence of different buffer concentrations are presented in Fig. 3a and b, respectively. In the presence of phosphate anions, calcium precipitates forming hydroxyapatite resulting in a reduction of free calcium ions (Figure S7) available to induce Ag NP aggregation. Under the same initial $\text{Ca}(\text{NO}_3)_2$ concentration, the concentration of Ca^{2+} remaining in solution decreases with the increase in buffer concentration (Figure S7) due to the increased precipitation of hydroxyapatite. To account for reduction in Ca^{2+} concentration on AgNP 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 3a-c). Fig. 3a-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-AgNPs. At the same counter ion concentration under RLA

regime, the attachment efficiency increases with the increase in buffer concentration (Fig. 3a-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 AgNP surface charge due to sorption of phosphate anions on the surface of AgNPs.

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-AgNP aggregation threshold (*ca.* > 40 mM Na^+ , Fig 3a) 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, Fig 3a). Therefore, the small concentrations of K^+ and Na^+ added from phosphate buffer and NaOH solutions cannot explain the differences in AgNP aggregation kinetics. Similarly, sequestration of Ca^{2+} ions is higher at higher buffer concentrations (Fig S7), resulting in more pronounced shift in the attachment efficiency curves when expressed as a function of Ca^{2+} remaining in solution (Fig 3c) compared to $\text{Ca}(\text{NO}_3)_2$ (Fig 3b). Thus, calcium sequestration by phosphate anions does not explain the differences in AgNP aggregation behavior. Consequently, the differences in AgNP aggregation behavior in the presence of phosphate buffer solution can be attributed to the higher adsorption of phosphate anions on the surface of cit-AgNPs at higher buffer concentrations, leading to a decrease in the surface charge (indicated by decrease in zeta potential) of cit-AgNPs at higher buffer concentrations (Fig. 4). Due to the low concentration of cit-AgNPs used in this study ($270.25 \mu\text{g L}^{-1}$), it was not possible to measure the zeta potential of AgNPs in the presence of buffer using laser Doppler electrophoresis. Therefore, to demonstrate the impact of buffer concentration on the surface charge of cit-AgNPs, we measured the zeta potential of cit-AgNPs at higher buffer concentrations (0.5 - 2.5 mM) and higher cit-AgNP concentration (5.4 mg L^{-1}). Cit-AgNPs 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-AgNPs, which can be attributed to the sorption/complexation of the phosphate anions on AgNP surfaces,^{52, 53} due to the high affinity of phosphate anions to silver ($K_{sp} = 8.89 \times 10^{-17} \text{ M}$).⁵⁴

The zeta potential of cit-AgNPs 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; Fig. 4). The zeta potential of AgNPs 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 AgNPs with phosphate anions at higher buffer concentrations.⁵⁵

The CCC as a function of phosphate buffer concentration decreases with the increase in buffer concentration (Fig. 5a and 5b), which can be attributed to the reduction in the zeta potential of AgNPs due to the sorption of phosphate anions on the surface of AgNPs. 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, Fig. 5a, 0.10 mM initial Ca(NO₃)₂, Fig. 5b, 0.017 mM free Ca²⁺, Fig. 5c). This behavior is in good agreement with the zeta potential reduction due to the sorption of phosphate anions on the surface of AgNPs (Fig. 4). 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 AgNPs surface with phosphate anions at higher buffer concentrations. The decrease in the CCC for citrate AgNPs with the increase in phosphate buffer concentration is in good agreement with the reported decrease in the CCC for charge stabilized-AgNPs with the increase in carbonate buffer concentration^{29, 30}. For instance, the CCC of Na⁺ for charge stabilized-AgNPs 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-AgNPs 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 AgNPs.⁵² Aggregation of phosphate coated AgNPs takes place through physical surface charge screening by counter ions (Na⁺ and Ca²⁺) according to DLVO theory.⁷

Aggregation impacted by fulvic acid

The UV-vis spectra of cit-AgNPs 10 minutes after interaction with phosphate buffer in the presence of 1 mgL⁻¹ SRFA show a decrease in the UV-vis absorbance at $\lambda = 396$ 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-AgNPs (Fig. 6, a-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 (Fig. S7a and b). For example, cit-AgNP peak at 396 nm in 1 mM buffer and

100 mM NaNO_3 decays to higher absorbance (~ 0.24 , Fig. S7a) in the presence of SRFA compared to the absence of SRFA (~ 0.08 , Fig. S7a) and the peak is broader in the absence of SRFA suggesting that SRFA increase the stability of cit-AgNPs against cation induced aggregation. Similarly, cit-AgNP peak at 396 nm in 1 mM buffer and 0.25 mM $\text{Ca}(\text{NO}_3)_2$ mM decays to higher absorbance (~ 0.30 , Fig. S7b) in the presence of SRFA compared to the absence of SRFA (~ 0.15 , Fig. S7b) and the peak is broader in the absence of SRFA suggesting that SRFA increase the stability of cit-AgNPs against cation induced aggregation. However, SRFA does not entirely eliminate the impact of phosphate anions on the aggregation of cit-AgNPs.

The attachment efficiency of cit-AgNPs in the presence of SRFA and at different phosphate buffer concentrations is presented in Fig. 7a and b for NaNO_3 and $\text{Ca}(\text{NO}_3)_2$. 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-AgNPs, presumably due to the sorption of phosphate anions on the surface of cit-AgNPs in the presence of SRFA. The CCC shifted to lower counter ion concentrations (157.7 ± 4.4 mM NaNO_3 and 1.5 ± 0.03 $\text{Ca}(\text{NO}_3)_2$) at higher buffer concentration compared to the CCC (265.3 ± 2.5 mM NaNO_3 and 1.8 ± 0.21 mM $\text{Ca}(\text{NO}_3)_2$) at lower buffer concentration (**Fig. 7 a and b, Table 1**). It is worth noting that in the case of Ca^{2+} counter ions (Fig. 7b), 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^{2+} concentration. This behavior can be attributed to inter-particle bridging by humic acid aggregates as observed elsewhere.²⁷ 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^{2+} ions.⁵⁶

Furthermore, at the same concentration of buffer, the CCC shifted to higher electrolyte concentration in the presence of SRFA (Fig. 7a and b) compared to the CCC values in the absence of SRFA (**Fig. 3 and Table 1**). It has been demonstrated that fulvic acid replaces citrate coating and form a surface coating on AgNPs and increase their stability.⁵⁷ Several studies have demonstrated the shift in CCC to higher counter ion concentrations in the presence of SRFA.^{1,27, 38} However, SRFA did not completely inhibit surface charge screening and NP aggregation by NaNO_3 and $\text{Ca}(\text{NO}_3)_2$ electrolytes.^{7, 58} Additionally, SRFA did not prevent the reduction in AgNP stability due to their interaction with phosphate anions.

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-AgNPs by NaNO_3 and $\text{Ca}(\text{NO}_3)_2$ by measuring the aggregation kinetics of cit-AgNPs 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-AgNPs used in this study ($270.25 \mu\text{g L}^{-1}$). The buffer concentration required to reach a stable CCC depends on the concentration of AgNPs, and decrease with the decrease in NP concentrations as lower buffer anions concentration will be required to fully coat the surface of AgNPs.

The majority of NP aggregation kinetic studies has focused on the role of counter ions (*ca.* Na^+ , Ca^{2+} , Mg^{2+})^{27, 59, 60}, and few studies investigated the impact of anions such as carbonates and phosphates on NP aggregation.^{61 7 62} Nonetheless, buffers (*e.g.* carbonate, phosphate, MOPS, *etc.*) are widely used in aggregation kinetic studies to maintain a constant pH.¹² These studies reported different CCC values. For instance, El Badawy *et al.* 2012²³ reported higher CCC (*ca.* 70 mM NaNO_3 and 5 mM $\text{Ca}(\text{NO}_3)_2$) for 58 nm cit-AgNPs compared to the study by Li 2011 (70 mM NaNO_3 and 5 mM $\text{Ca}(\text{NO}_3)_2$) for 64 nm cit-AgNPs²⁶. Based on our findings, the lower CCC values reported in Li *et al.* (2011) compared to those reported by Badawy *et al.* 2012²³ can be attributed (at least partially) to the higher carbonate buffer concentration used in the former study.

Considering the lower expected environmental concentrations of AgNPs ($0.01\text{-}10 \mu\text{g L}^{-1}$)^{4, 5} compared to those investigated in this study ($270.25 \mu\text{g L}^{-1}$), it is likely that at lower NP concentrations, the CCC will tend to a constant value at lower phosphate anion concentrations as lower phosphate anion concentrations will be required to saturate the surface of AgNPs. Considering the higher NP concentrations typically studied in the literature *e.g.* (mg L^{-1} ; Table S1), it is likely that studies performed at high NP concentration underestimates the attachment efficiencies and CCC, and these values need to be considered carefully.

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List of abbreviations

NPs: nanoparticles

AgNPs: silver nanoparticles

SRFA: Suwannee River Fulvic Acid

CCC: critical coagulation concentration

α : attachment efficiency

W: stability ratio

RLA: reaction limited aggregation

DLA: diffusion limited aggregation

NOM: natural organic matter

DLS: dynamic light scattering

EPM: electrophoretic mobility

Fig. 1. UV-vis spectra of cit-AgNPs (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

Fig. 2. Evolution of the UV-vis absorbance at λ_{396} of cit-AgNPs ($270.25 \mu\text{g L}^{-1}$) as a function of time immediately after mixing with different concentrations of NaNO₃ (a and b) and Ca(NO₃)₂ (c and d) in different concentrations of buffer (pH 7.0): (a, and c) 0.05 mM, and (b, and d) 1 mM

Fig. 3. Attachment efficiency of cit-AgNPs ($270.25 \mu\text{g L}^{-1}$) at different concentrations of phosphate buffer reacted with: (a) NaNO_3 , (b) $\text{Ca}(\text{NO}_3)_2$, and (c) Ca^{2+} remaining in solution. All experiments were performed at pH 7.0. Figure legend represents potassium phosphate monobasic buffer concentration

Fig. 4. Zeta potential of cit-AgNPs 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.

Fig. 5. Critical coagulation concentration of cit-AgNPs (270.25 $\mu\text{g L}^{-1}$) as a function of phosphate buffer concentration in the presence of monovalent and divalent electrolytes (a) NaNO_3 , (b) $\text{Ca}(\text{NO}_3)_2$ and (c) Ca^{2+} remaining in solution following precipitation of hydroxyapatite. Dotted line is used to guide the reader eye.

Fig. 6. UV-vis spectra of cit-AgNPs ($270.25 \mu\text{g L}^{-1}$) 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 $\text{Ca(NO}_3)_2$ (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

Fig. 7. Attachment efficiency of cit-AgNPs ($270.25 \mu\text{g L}^{-1}$) at different concentrations of phosphate buffer reacted with: (a) NaNO_3 , (b) $\text{Ca}(\text{NO}_3)_2$ in the presence of SRFA (1 mg L^{-1})

Table 1. CCC values in the presence and absence of SRFA

Buffer Concentration (mM)	Electrolyte	CCC in the presence of SRFA (mM)	CCC in the absence of SRFA (mM)
0.05	NaNO_3	265.3 ± 2.5	232.0 ± 1.4
1.00	NaNO_3	157.7 ± 4.4	90.9 ± 2.2
0.05	$\text{Ca}(\text{NO}_3)_2$	1.8 ± 0.21	0.7 ± 0.001
1.00	$\text{Ca}(\text{NO}_3)_2$	1.5 ± 0.03	0.1 ± 0.008

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