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**Citrate-coated silver nanoparticles interactions with effluent organic
matter: influence of capping agent and solution conditions**

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Abstract: Fate and transport studies of silver nanoparticles (AgNPs) discharged from urban wastewaters containing effluent organic matter (EfOM) into natural waters represent a key knowledge gap. In this study, EfOM interfacial interactions with AgNPs and their aggregation kinetics were investigated by atomic force microscopy (AFM) and time-resolved dynamic light scattering (TR-DLS), respectively. Two well-characterized EfOM isolates, i.e., wastewater humic (WW humic) and wastewater colloids (WW colloids, a complex mixture of polysaccharides-proteins-lipids), and a River humic isolate of different characteristics were selected. Citrate-coated AgNPs were selected as representative capped-AgNPs. Citrate-coated AgNPs showed a considerable stability in Na^+ solutions. However, Ca^{2+} ions induced aggregation by cation bridging between carboxyl groups on citrate. Although the presence of River humic increased the stability of citrate-coated AgNPs in Na^+ solutions due to electrosteric effects, they aggregated in WW humic-containing solutions, indicating the importance of humics characteristics during interactions. Ca^{2+} ions increased citrate-coated AgNPs aggregation rates in both humic solutions, suggesting cation bridging between carboxyl groups on their structures as a dominant interacting mechanism. Aggregation of citrate-coated AgNPs in WW colloids solutions was significantly faster than those in both humic solutions. Control experiments in urea solution indicated hydrogen bonding as the main interacting mechanism. During AFM experiments, citrate-coated AgNPs showed higher adhesion to WW humic than to River humic, evidencing a consistency between TR-DLS and AFM results. Ca^{2+} ions increased citrate-coated AgNPs adhesion to both humic isolates. Interestingly, strong WW colloids interactions with citrate caused AFM probe contamination (nanoparticles adsorption) even at low Na^+ concentrations, indicating the impact of hydrogen bonding on adhesion. These results suggest the importance of solution conditions and capping agents on the stability of AgNPs in solution. However, the characteristics of

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organics would play a crucial role in the fate and transport of these nano contaminants in
urban wastewaters and natural water systems.

1. Introduction

Engineered silver nanoparticles (AgNPs) are at present widely used for numerous commercial, industrial, and biotechnological applications (e.g., biological markers and probes, catalysis, photonic materials, coatings).^{1, 2, 3, 4} In addition, due to their antimicrobial and antiviral properties, AgNPs have attracted considerable attention in medical fields where they have become a crucial material.⁵ Nowadays, approximately 320 tons/year of nanosilver are globally produced, evidencing its proliferation.⁶ Nevertheless, the release of these AgNPs in the environment and their possible chemical/physical transformations (i.e., sulfidation, dissolution, precipitation, aggregation)⁷ would cause adverse effects (e.g., toxicity, inhibitory effects)^{8, 9} on microbial communities, ecosystems, and humans. Therefore, fate and transport of AgNPs in the environment and specially their interactions with other components of water systems have become a topic of high debate among scientific circles and regulators.

Extensive research has been conducted on AgNPs characteristics (e.g., surface charge, size, capping agents)^{10, 11, 12} that would influence their transport over large distances. Similarly, AgNPs interactions with individual and ever present components of natural waters (e.g., natural organic matter, inorganic ions, etc.) have also been studied using varied sensitive techniques (e.g., Fluorescence Correlation Spectroscopy, Dynamic Light Scattering, Atomic Force Microscopy, and Asymmetrical Flow Field-Flow Fractionation). These previous studies have observed that bare AgNPs readily aggregate at very low salt concentration in solution (i.e., Critical Coagulation Concentration $CCC < 10$ mM NaCl and $CCC < 1$ mM $CaCl_2$).¹³ Ca^{2+} was suggested to more efficiently neutralize bare AgNPs surface charge and thus to induce faster aggregation than K^+ or Na^+ at similar ionic strength.¹⁰ These results suggest a high bare AgNPs instability in natural waters. Nevertheless, to prevent aggregation during AgNPs preparation, capping agents are widely utilized.⁷ Polyvinyl pyrrolidone (PVP) and citrate are commonly used capping agents due to their high chemical stability and high

solubility in polar solvents.^{4, 12} Citrate is a small molecular weight chelating agent (carboxylic acid) that increases the magnitude of the negative charge on AgNPs surface; while PVP is an uncharged polar molecule suggested as one of the most efficient polymers for AgNPs stabilization. PVP and citrate induce steric and electrostatic repulsive forces, respectively, that significantly reduce aggregation and deposition to surfaces. However, these capping agents would be also expected to influence AgNPs surface charge, aggregation/deposition behavior, and associations with other components of natural waters. For instance, compared to bare AgNPs, citrate-coated AgNPs have shown a considerably higher stability in natural fresh waters due to electrostatic colloidal stabilization.¹¹ However, a high concentration of ions (e.g., seawater) or the presence of divalent cations are needed to break citrate-coated AgNPs stability by means of charge screening and cation bridging mechanisms, respectively.^{11, 14} On the other hand, PVP-coated AgNPs have shown more stability than citrate-coated AgNPs in both monovalent and divalent cation solutions due to steric mechanisms.¹⁴ Interestingly, the presence of humic acid in solution promoted even more the stability of both PVP-coated and citrate-coated AgNPs as evidenced by their higher CCC, and also further decreased their deposition to silica surfaces by hindering attachment.^{12, 14} Similarly, the adsorption of different surface water humic fractions (i.e., Suwannee River Humic/Fulvic Acid, Lake Hohlo Humics, etc.) to bare AgNPs induced stability (i.e., and even caused disaggregation) by steric repulsive forces and also increased the magnitude of bare AgNPs negative charge.^{15, 16, 17, 18} Overall, what is clear from these previous studies is that (capped) AgNPs readily interact with other components of natural surface waters and that a high mobility under environmentally relevant solution conditions would be expected.

Nevertheless, there are still open questions and needed research regarding the environmental scenario of AgNPs released from domestic/industrial sources, entering sewer systems, and

eventually reaching wastewater treatment plants. Specifically, capped-AgNPs associations with Effluent Organic Matter (i.e., EfOM, organic compounds from urban wastewater) are currently unknown. Understanding the influence of capping agents during AgNPs interactions with EfOM is crucial for understanding their fate, transport, and impact to ecosystems when discharged to aquatic environments. Thus, the first objective of this study was to investigate capped-AgNPs aggregation and interfacial interactions with selected EfOM isolates under different solution conditions. The second objective was to elucidate the interacting mechanisms between capped-AgNPs and EfOM isolates as a function of their physicochemical characteristics. For the current study, citrate-coated AgNPs were selected as representative capped-AgNPs; while a humic EfOM and a colloids EfOM fraction were selected as WW organic isolates. Also, a River humic NOM was selected to conduct a comprehensive comparison with the EfOM fractions. Aggregation of capped-AgNPs was studied by Time-Resolved Dynamic Light Scattering (TR-DLS). Atomic Force Microscopy (AFM) was used to investigate interfacial interactions. Due to its sub-nano Newton resolution, AFM is a versatile tool to probe interaction forces at the nanometer-scale under varied solution chemistries.

2. Experimental

2.1. Reagents and Solution Chemistries

Ultrapure water (resistivity of 18.2 MΩ.cm, Millipore, USA) was used to prepare all electrolyte solutions and colloidal suspensions. Solutions were prepared before use with analytical grade reagents (e.g., NaNO₃, NaOH, NaCl, CaCl₂, etc.), and then filtered through a 0.22 μm membrane. Iron Oxide solution used for layer-by-layer coating procedure was prepared by increasing the pH of a 10 mM FeCl₃ solution to 7, as previously described.¹⁹ Citrate-coated AgNPs were acquired in diameter of 60 nm (Nanocomposix, USA) and were originally stabilized in 2 mM citrate per the manufacturer. This stock was stored at 4°C in the

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3 dark until use. A diafiltration process in a 1 mM NaNO₃ solution using an Amicon cell
4 (Millipore, USA) was conducted for removing colloidal citrate from the citrate-coated AgNPs
5 stock. After purification, the final concentration of the citrate-coated AgNPs suspension was
6 20 ppm. The characteristics of the nanoparticles suspension were constantly monitored by
7 electrophoretic mobility and hydrodynamic diameter measurements, where no significant
8 change was observed for the duration of the study. Dissolution experiments were conducted
9 for measuring the dissolved silver concentration of the citrate-coated AgNPs suspension and
10 of citrate-coated AgNPs in solution at the highest electrolyte concentration tested (i.e., 100
11 mM NaCl) using an Inductively Coupled Plasma Mass Spectrometry equipment (ICP-MS,
12 APHA method 3125). The dissolved silver concentrations obtained (i.e., 0.0013 and 0.0077
13 mg/L, respectively) were considerably low. Simulations conducted by Visual MINTEQ V3.0
14 indicated that no precipitation of any silver species would occur at equilibrium. This result
15 suggested that citrate-coated AgNPs aggregation would not be influenced by the dissolution
16 of silver or inter-nanoparticle bridging by precipitated silver salts.
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34 **2.2. Effluent Organic Matter isolates: origin, characteristics, and stock preparation**

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36 The EfOM fractions were isolated from treated wastewater (secondary effluent) obtained
37 from Jeddah wastewater treatment plant (Al Ruwais district, Jeddah, Saudi Arabia) and were
38 rigorously characterized in a previous investigation.²⁰ Briefly, WW humic (i.e., hydrophobic
39 acids) was found rich in carboxylic and aromatic ring structures. The presence of sulfonic
40 groups associated to carboxyl moieties was also detected in this fraction, while signals of
41 proteins or carbohydrates were almost absent in ¹³C-NMR and FT-IR spectra. Specific signal
42 attributed to aromatic aminoacids were, however, observed from the fluorescence excitation
43 emission spectrum. Conversely, WW colloids (i.e., hydrophilic colloids) showed a high
44 organic nitrogen content and a weak presence of carboxyl and aromatic ring structures. Also,
45 a large presence of proteins, aminosugars, and polysaccharides was observed in this fraction.
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Indeed, carbohydrates constituted a major part of this isolate. These characteristics confer WW colloids a stronger hydrophilic character compared to WW humic. The selected River humic isolate (Gartempe river-hydrophobic acid)²¹ presented typical surface water humic substance characteristics, i.e., a predominance of fulvic acid structures incorporating aromatic/phenolic carbon and carboxyl groups. The organic matter (OM) stock solutions were prepared by dissolving the selected OM isolate in 1 mM NaCl solution, to an approximate final concentration of 200 mg C/L.¹⁹ The OM solution was then stirred overnight, filtered through a 0.45 μm membrane, and stored at 4°C in the dark until use. The Total Organic Carbon concentration of OM stock solutions was measured using a TOC-V CPH total organic carbon analyzer (Shimadzu, Japan).

2.3. Electrophoretic Mobility (EPM) measurements of organic matter isolates

A ZS ZEN3600 Zetasizer (Malvern, UK) and 1 mL clear disposable zeta cells (DTS1060C, Malvern, UK) were used to measure the EPM of WW humic, WW colloids, and River humic. Briefly, 1.6 μm in diameter silica particles (PolySciences, USA) were coated with the selected OM isolate following the layer-by-layer protocol and using iron oxide as an intermediate layer, as previously described.¹⁹ The EPM measurement of the OM-coated particles were conducted in 1, 3, 10, 30, and 100 mM Na⁺ solutions, and 0.33, 1, 3.33, 10, and 33 mM Ca²⁺ solutions at ambient pH. A minimum of 3 measurements per sample were conducted for every electrolyte solution at room temperature (25°C) using Dispersion Technology Software (v5.10, 2008, Malvern, UK). The data sets were pooled and averaged.

2.4. Determination of citrate-coated AgNPs aggregation kinetics in OM-containing electrolyte solutions by Time-Resolved Dynamic Light Scattering (TR-DLS)

The initial hydrodynamic diameter (D_{ho}) of citrate-coated AgNPs was measured immediately before every experiment using a ZS ZEN3600 Zetasizer (Malvern, UK) and low volume plastic disposable cuvettes (ZEN112, Malvern, UK). Citrate-coated AgNPs solutions were

prepared by diluting the stock suspensions in a 1 mM NaNO₃ solution to a final concentration of 1 ppm and to a final volume of 500 μL. Second-order cumulant analysis by Dispersion Technology Software (v5.10, 2008, Malvern, UK) were used to determine the corresponding intensity-weighted hydrodynamic diameter of citrate-coated AgNPs. A minimum of three measurements per solution condition were conducted where the data sets were pooled and averaged. This D_{h0} served as a baseline measurement for the following aggregation kinetics experiments. First, TR-DLS technique was used to determine the aggregation kinetics of citrate-coated AgNPs in electrolyte solutions. In every experiment, citrate-coated AgNPs were added to an electrolyte solution to a final concentration of 1 ppm and to a final ionic strength ranging from 1-100 mM Na⁺ or Ca²⁺. After a gentle shake, the cuvette was immediately placed into the Zetasizer instrument for measurement. Autocorrelation functions were accumulated for 20 seconds, and the measurements extended over 1 hour to allow an increase in the intensity hydrodynamic diameter (D_h) by 38% of the initial hydrodynamic diameter (D_{h0}) as previously described.²² Next, the aggregation kinetics of citrate-coated AgNPs in OM-containing solutions were measured in 1, 3, 10, 30, 50, and 100 mM Na⁺, and 0.33, 1, 3.33, 10, 16.6, and 33 mM Ca²⁺. Due to limited amount of OM sample, TR-DLS experiments and replicates were only conducted on these solution conditions (i.e., laborious procedures and large volumes of water are required to isolate various organic fractions of different structural properties).²³ Similarly to the previous experimental condition, citrate-coated AgNPs and OM stock solutions were simultaneously added to the selected electrolyte solution at a final concentration of 1 ppm and 15 mg C/L, respectively, gently shook, and placed in the Zetasizer equipment for immediate measurement. The experimental aggregation kinetic rate constant (k₁₁) of citrate-coated AgNPs in electrolyte solution or in OM-containing electrolyte solution was determined as the initial rate of increase in the nanoparticles hydrodynamic diameter (D_h) as a function of time (t). Specifically, k₁₁ is determined as the

initial slope of the D_h vs time plot up to the point at which D_h reaches the relative hydrodynamic diameter of $1.38D_{h0}$, as previously described.^{22, 24} However, since this aggregation rate is inversely proportional to the initial concentration of nanoparticles (N_0),¹⁴ citrate-coated AgNPs concentration in solution (i.e., 1 ppm) was maintained fixed throughout this study for conducting a consistent comparison between all the experimental conditions. In the current investigation, k_{11} was expressed in units of L/t (i.e., nm.min⁻¹), following: $k \propto (dD_h(t)/dt)_{t \rightarrow 0}$, and considering N_0 as constant for all experimental conditions.¹⁴ Inverse stability ratios (i.e., attachment efficiencies) were not calculated and reported because a clear favorable aggregation region (k_{fast}) could not be observed in some experimental conditions. In addition, control experiments were performed to measure the aggregation rates of the OM isolates (15 mg C/l) in solutions ranging from 1-100 mM Na⁺ as previously described.²² No OM aggregation (i.e., River humic, WW humic, or WW colloids) was detected in solutions containing up to 100 mM Na⁺ (Figure S1a). This stability of OM molecules in Na⁺ solutions assisted in the elucidation of citrate-coated AgNPs interacting mechanisms with OM fractions. Similar hydrodynamic diameters of organic molecules have been previously reported by DLS and Fluorescence Correlation Spectroscopy.^{22, 25}

2.5. Measurement of interaction forces between citrate-coated AgNPs and OM isolates

Interaction forces between OM-coated AFM colloidal probe and citrate-coated AgNPs-coated substrate were measured using a 5500 Scanning Probe Microscope (5500 SPM, Agilent Technologies, USA). The OM-coated AFM colloidal probes were prepared as follows.¹⁹ Briefly, a 20 μ m silica sphere was glued to a cantilever of 0.3 N/m spring constant (AppNano, USA). The spring constant of the colloidal probes were measured by the thermal tuning method,²⁶ where no significant deviation from the manufacturer specification was observed. The OM coating of the colloidal AFM probes followed the layer-by-layer procedure.¹⁹ First, a colloidal AFM probe was immersed in 300 μ L of iron oxide solution

and allowed to coat for 12 hours. Then, the iron oxide-coated probe was rinsed in ultrapure water and immersed in the selected OM solution (i.e., at a concentration of 50 mg C/L) and allowed to coat for 12 hours. Finally, the OM-coated colloidal probe was rinsed in ultrapure water and immediately used for experimentation. As a control experiment, the spring constants of the OM-coated colloidal probes were measured and compared to those of bare colloidal probes, where no significant deviation was detected. Micrographs obtained by a Quanta 3D-FEG SEM equipment (FEI, Netherlands) were used to assess the surface modification of the colloidal probes by OM coating as described elsewhere.¹⁹ The preparation of citrate-coated AgNPs-coated substrates followed the protocol previously described.²² Approximately 250 μ L of iron oxide solution was pipetted onto a previously cleaned, smooth silica surface (roughness~2.5 nm) of approximately 0.4 cm² in surface area and allowed to coat for 12 hours. The iron oxide coated surface was rinsed with ultrapure water and then citrate-coated AgNPs stock solution was pipetted onto the iron oxide-coated substrate and left undisturbed for 12 hours. Finally, the substrate was carefully rinsed with ultrapure water and immediately used for experimentation. Similar approaches taking advantage of electrostatics have been successfully conducted for surface modification.²⁷ Topographic images generated by AFM were used to assess the citrate-coated AgNPs-coating completeness of silica substrates by scanning the surface in tapping mode with a sharp silica AFM tip (k : 0.3 N/m, radius of curvature: ca. 10 nm, AppNano, USA) and in 1 mM NaNO₃ solution. In addition to topographic profiles, root mean squared surface roughness (R_{RMS}) was determined. Force vs. distance profiles were acquired in contact mode at a 500 nm/s rate, consistent with previous studies,²⁸ and using Pico View v.1.8.2 software (Agilent Technologies, USA). A minimum of 40 force profiles were recorded for every experimental and solution condition at different locations of the substrate of analysis. Retraction force profiles were individually analyzed for maximum adhesion force and adhesion energy. Specifically, maximum adhesion forces are

defined as the maximum force recorded before total OM-coated probe detachment from the citrate-coated AgNPs-coated surface. The adhesion forces were normalized to the radius of the colloidal probe.²⁶ Adhesion energies were calculated by integrating the adhesion forces with the separation distances using the trapezoidal rule.²⁹ Maximum adhesion forces and adhesion energies were processed by probability density functions where mean and variance were determined. The results obtained from this statistical analysis were correlated to the characteristics of the OM isolates and citrate-coated AgNPs to elucidate their dominant interacting mechanisms. Immediately after AFM experiments, the OM-coated AFM probes were subjected to scanning electron microscope analysis to detect any possible probe contamination (i.e., adsorption of citrate-coated AgNPs on the OM layer).

3. Results and Discussion

3.1. Electrophoretic Mobility Measurements

Solution chemistry had a clear effect on the surface charge of all the samples. The EPM of River humic, WW humic, and WW colloids were negative throughout the entire range of Na^+ concentration tested (Figure 1a). This negative charge would be the results of ionized carboxyl groups present on the structure of the OM samples^{20, 21, 30} as evidenced in previous characterization studies.^{20, 21} In addition, with increasing Na^+ concentration, the EPM of the three OM samples became less negative, suggesting charge screening (i.e., compression of diffuse double layer) due to interactions with indifferent Na^+ cations. River humic and WW humic displayed a considerably higher negative charge than WW colloids at every solution condition tested due to their higher abundance of carboxyl groups in their structures,^{20, 21} and were consistent with previous investigations.¹⁹ In Ca^{2+} solutions, the charge of the three OM isolates were significantly reduced compared to those in Na^+ solutions (Figure 1b). However, the charge of the humic samples were more impacted by Ca^{2+} cations. This observation indicates the influence of Ca^{2+} in the surface charge of OM molecules possibly through

complexation with deprotonated carboxyl groups.³⁰ The magnitude of EPM values followed the order of River humic>WW humic>WW colloids at every solution condition tested.

3.2. Aggregation kinetic of citrate-coated AgNPs in OM-containing electrolyte solutions

The stability of citrate-coated AgNPs was studied under a wide range of solution chemistries and in the absence and presence of dissolved OM. The average D_h of citrate-coated AgNPs in solution measured before experiments was 68.5 ± 2.6 nm. The mean polydispersity index (PDI) of the cumulant analysis was <0.15 , indicating a monodispersed population. In Na^+ solutions of low ionic strength (1 mM) and in zero salt solutions (i.e., conducted as a control), citrate-coated AgNPs remained stable against aggregation over the 1 hour experimental window (Figure S1b); both conditions showed statistically similar hydrodynamic diameters (t-test, $p = 0.05$). Nevertheless, a clearer signal of aggregation was detected at 10 mM Na^+ , and increased with increasing Na^+ in solution (Figure 2a). In previous studies, citrate-coated AgNPs have displayed a significantly higher stability in solution than bare AgNPs due to electrostatic colloidal stabilization by citrate (i.e., small MW chelating agent comprised of carboxyl acid that increases the magnitude of the negative charge on the AgNPs surface).¹¹ In contrast, aggregation of citrate-coated AgNPs was observed at 0.33 mM Ca^{2+} solutions (Figure 2a and S1c) and also increased with increasing Ca^{2+} in solution. However, at 10, 16.6, and 33.3 mM Ca^{2+} the aggregation rates remained approximately constant, clearly indicating a favorable aggregation (i.e., diffusion limited regime).³¹ Previous investigations have similarly reported that a high concentration of ions or the presence of divalent cations is needed to break the stability in solution of citrate-coated AgNPs by means of charge screening or cation bridging occurring between ionized carboxyl groups on citrate molecules, respectively.^{11, 14} Interestingly, the presence of River humic slightly decreased the aggregation rates of citrate-coated AgNPs in Na^+ solutions (Figure 2a). For instance, the

aggregation rates of citrate-coated AgNPs at 50 mM Na⁺ and in the absence or presence of River humic were 0.58 and 0.14 nm.min⁻¹, respectively. Moreover, the aggregation rates at 100 mM Na⁺ and in the absence or presence of River humic were 1.31 and 0.81 nm.min⁻¹, respectively. Previous studies have similarly observed that the presence of humic acid (i.e., of surface water origin) in solution enhanced the stability of citrate-coated AgNPs (i.e., increasing CCC) and even decreased their deposition on silica surfaces by hindering attachment.^{12, 14} Other studies have reported that the adsorption of different humic isolates (i.e., Suwannee River humic/Fulvic Acid, Lake Hohlo Humics, etc.) to bare AgNPs induced stability (i.e., and even caused disaggregation) by means of steric repulsive forces and also by increasing the magnitude of the negative charge of bare AgNPs.^{15, 16, 17, 18} Consequently, strong electrostatic and steric interactions between citrate and River humic would cause these low interactions in Na⁺ solutions, i.e., also evidenced in zero salt solutions (Figure S1b). In contrast to their high stability in the presence of Na⁺, citrate-coated AgNPs readily aggregated in Ca²⁺ and River humic-containing solutions (Figure 2a). Aggregation in this system was observed at 1 mM Ca²⁺. The aggregation rates increased with increasing Ca²⁺ concentration, indicating other aggregation mechanisms (e.g., heteroaggregation and bridging). However, at the maximum Ca²⁺ concentration tested (i.e., 33 mM), no favorable aggregation (i.e., diffusion limited regime) was observed. The aggregation rate constants of citrate-coated AgNPs in River humic-containing solution and at 10, 30, 50, and 100 mM Na⁺ were above 2 orders of magnitude lower than in Ca²⁺ solutions of similar ionic strength. As previously suggested, Na⁺ ions act as indifferent cations while Ca²⁺ ions form inner-sphere complexes with deprotonated carboxyl groups, thus more efficiently screening charges and decreasing energy barriers. These results evidence the high interactions occurring between Ca²⁺ ions and ionized carboxyl groups on both citrate and River humic OM.^{30, 32, 33}

Citrate-coated AgNPs aggregation in WW humic-containing solutions was clearly observed at 10 mM Na⁺ with a k_{11} of 0.28 nm.min⁻¹, (Figure 2b and S1d). Furthermore, the aggregation rate constant increased at 20 and 30 mM Na⁺, indicating reaction limited regime. However, this value remained approximately similar at 30, 50, and 100 mM Na⁺ (i.e., 2.09, 2.75, and 2.79 nm.min⁻¹, respectively, Figure S1d), evidencing a region favorable aggregation. This aggregation behavior was markedly dissimilar to that in River humic-containing solutions and suggests high interactions between WW humic and citrate even at low Na⁺ concentrations, where the characteristics of WW humic would play a key role. A previous rigorous characterization showed that WW humic and River humic are rich in carboxylic and aromatic ring structures.²⁰ However, a larger presence of sulfonic groups associated to carboxyl moieties (i.e., possibly derived from detergents) and also a larger content of lipids/fatty acids (i.e., clearly identified from FT-IR spectra and possibly originating from strong biological activity in aerobic sludge reactors) were detected in WW humic. Additionally, the SUVA₂₅₄ of WW humic (i.e., a good indicator of the abundance of aromatic moieties in complex organic matrices) was calculated as 2.9, data that is in the upper level of the range of values determined for wastewater humic fractions.^{34, 35} Conversely, higher SUVA₂₅₄ values have been observed for humic material isolated from high DOC/highly colored surface waters (e.g., 4.0 and 4.8 for Gartempe River and Suwannee River NOM, respectively).¹⁹ This moderate WW humic SUVA₂₅₄ value would indicate a different aromatic character as compared to River humic.³⁴ Remarkably, although both River humic and WW humic fractions were isolated using the same protocol, they have distinctive characteristics that would influence their interactions with citrate-coated AgNPs. In addition, the lower EPM of WW humic compared to that of River humic (Figure 1a) would result in lower electrostatic repulsive forces to citrate-coated AgNPs. Electrostatic forces are proportional to the product of the charges between the selected OM isolate and citrate-coated

AgNPs, following Coulomb's law.³⁶ Consequently, a lower double layer interaction (i.e., and reduced energy barrier) would be expected between WW humic and citrate than between River humic and citrate. Nevertheless, the elucidation of the precise dominant interacting mechanism influencing WW humic association with citrate-coated AgNPs as a function of WW humic characteristics (i.e., degree of aromaticity, presence of sulfonic groups, content of lipids/fatty acids, etc.) is beyond the scope of this study and would require additional extensive investigation. Furthermore, the presence of divalent cations exerted a higher influence on the aggregation of citrate-coated AgNPs in WW humic solutions than monovalent cations (Figure 2b). Specifically, the aggregation rate constants in Ca^{2+} solutions were above one order of magnitude higher than those in Na^+ solutions at similar ionic strength (K_{11} : 5.7, 29.4, 32.6, and 35.2 $\text{nm}\cdot\text{min}^{-1}$ at 3.33, 10, 16.6, and 33.3 mM Ca^{2+} , respectively). This faster aggregation would be caused by a more efficient charge neutralization by Ca^{2+} on deprotonated carboxyl groups on citrate and WW humic structures. Carboxyl groups are a dominant functional group on WW humic and previous studies on Molecular Dynamic have suggested high interactions between these ionized groups on NOM and divalent cations.^{20, 30, 32, 33} Furthermore, CCC in Ca^{2+} solutions would occur at a lower ionic strength than in Na^+ solutions. Nevertheless, due to sample volume constraints it was not possible to exactly determine the critical coagulation concentration to establish an accurate comparison between favorable and unfavorable aggregation in Na^+ or Ca^{2+} solutions.

The aggregation behavior of citrate-coated AgNPs in WW colloids solution radically differed from that in River humic or WW humic solutions. A quick aggregation was detected at as low as 1 mM Na^+ solution (Figure 2b). These nanoparticles aggregated faster with increasing ionic strength (K_{11} : 7.5, 10.7, 23.2, 44.2, and 70.8 $\text{nm}\cdot\text{min}^{-1}$ at 1, 3, 10, 30, and 100 mM Na^+ , respectively). However, even at the highest ionic strength tested (100 mM Na^+) no clear

region of favorable aggregation was detected. The aggregation rate constants (k_{11}) of citrate-coated AgNPs in WW colloids-containing solutions were higher than in WW humic or in River humic-containing solutions at every Na^+ concentration tested. The characteristics of WW colloids would play a key influence during interactions with citrate. WW colloids, incorporating significantly larger molecular structures (retained in 3,500 Da dialysis bag) than humic material (permeate through 3,500 Da dialysis bag), has been characterized as a hydrophilic fraction showing a high organic nitrogen content and a weak presence of carboxyl and aromatic ring structures (i.e., high content of aliphatic structures). This fraction is also comprised of proteins, aminosugars, and polysaccharides (i.e., carbohydrates constitute a major part of this isolate). This characteristics explain the low charge of this isolate compared to the humic samples. Specifically, this low charge would also allow lower repulsive forces (i.e., lower energy barriers) to citrate and higher charge screening with increasing ionic strength in solution (Figure 1a). In addition, possible short-ranged hydrogen bonding mechanisms would be occurring between ionized carboxyl groups on citrate and alcohol groups on WW colloids (i.e., dominant functional group on WW colloids structure). To test this latter hypothesis, the following control experiment was conducted. The aggregation rate of citrate-coated AgNPs was measured in WW colloids-containing solution at 1 mM Na^+ , in the presence and absence of urea. The high ability of urea to form hydrogen bonds based on their two proton-donating groups (N-H) and one proton-accepting group (C=O), has been widely documented (e.g., denaturing proteins).³⁷ As a chaotropic agent, urea would be expected to disrupt hydrogen bonding networks between WW colloids and citrate. Results from this control experiment show that urea affected WW colloids associations with citrate-coated AgNPs (i.e., influencing aggregation) and therefore reinforced the hypothesis of hydrogen bonding as the dominant interacting mechanism in this system (Figure S2). Similar to WW humic and River humic, the presence of Ca^{2+} also influenced the aggregation

behavior of citrate-coated AgNPs in WW colloids solution (Figure 2b). The aggregation of these nanoparticles was faster with increasing Ca^{2+} concentration (K_{11} : 18.6, 41.2, 52.5, 68.9, and 116.6 $\text{nm}\cdot\text{min}^{-1}$ at 0.33, 1, 3.33, 10, and 33.3 mM Ca^{2+} , respectively), suggesting a more efficient charge screening and possible cation bridging mechanism between citrate and carboxyl groups on WW colloids. However, the aggregation rate constant in Ca^{2+} solution was slightly higher than in Na^{+} solution at low salt concentrations (i.e., specifically, in the same order of magnitude). This smaller difference in aggregation rate constants compared to the humic samples, would be explained by the lower presence of carboxyl groups on WW colloids structure. Additionally, short-ranged hydrogen bonding mechanisms would be expected to play a dominant role during citrate-WW colloids interactions as observed in the urea control experiment. Also, no region of favorable aggregation was clearly detected in the Ca^{2+} concentration range tested. Finally, due to the larger molecular structure of WW colloids compared to those of humic fractions, a possible hetero-aggregation mechanism with AgNPs significantly increasing the aggregation rate constants would also occur.

3.3. Interfacial interactions between citrate-coated AgNPs and OM isolates

The AFM images in tapping mode of smooth silica wafer (i.e., used as a substrate for subsequent nanoparticle deposition) and citrate-coated AgNPs adsorbed on a smooth silica wafer provided a direct evidence of complete coverage of the substrate (Figure 3a-b). The average diameter of the citrate-coated nanoparticles (i.e., determined from height measurements) was calculated as 64.3 ± 2.9 nm ($n=75$). Nevertheless, the nanoparticle-coated substrate revealed a complex morphology at the nanoscale. The adsorbed layer of nanoparticles showed physically heterogeneous, and were not arranged as a perfect monolayer. The Root Mean Squared Roughness (R_{RMS}) of the adsorbed nanoparticle layer was calculated as 26.4 nm (area: $0.25 \mu\text{m}^2$). Furthermore, no visible voids or gaps were detected in the nanoparticle-coated substrate.

Maximum adhesion forces and adhesion energies between OM fractions and citrate-coated AgNPs were analyzed at different solution conditions. These force profiles provided crucial information about the type of interaction and strength of adhesion. River humic maximum adhesion force to citrate-coated AgNPs in Na^+ solutions were small in magnitude (Figure 4a, S3a-c, S11), and slightly increased with increasing Na^+ concentration (i.e., 0.01, 0.02, and 0.09 mN/m at 1, 10, and 100 mM Na^+ , respectively). Similarly, their adhesion energies (Figure 5a, S4a-c) also increased with increasing salt in solution. These low adhesion forces and low adhesion energies would be caused by steric interactions due to the polymeric nature of this humic sample,^{19, 38} in addition to electrostatic repulsive forces displayed by both highly negatively-charged citrate and River humic. However, the presence of Ca^{2+} radically change the force profiles. First, the retracting curves showed multiple detachment events, which have been observed in previous studies and would be attributed to complex multiple discrete OM adsorption on rough surfaces (Figure S11).²² The maximum adhesion forces significantly increased in comparison to those in Na^+ solutions. In addition, these forces increased in magnitude with increasing Ca^{2+} concentration (Figure 4b, S5a-c). These results evidenced the impact of Ca^{2+} during citrate interactions with River humic, and would be caused by cation bridging between deprotonated carboxyl groups on both structures. Likewise, the adhesion energies in Ca^{2+} were also considerably higher than those in Na^+ solutions (Figure 5b, 6a-c). These results are in good agreement with the citrate-coated AgNPs aggregation experiments in River humic-containing solutions. Furthermore, it is worth noticing that the statistical analysis of maximum adhesion forces and adhesion energies was conducted by probability density functions due to scatter in the data. This scatter would be caused by physical heterogeneities on the nanoparticle-coated substrate, and also by physicochemical heterogeneities on the organic matter sample.³⁹ In previous studies, this

scatter in the data was not observed during organic interactions with mica,¹⁹ possibly due to the low roughness and chemical homogeneity of the latter.

In contrast, WW humic maximum adhesion forces and adhesion energies to citrate-coated AgNPs in Na⁺ solution were considerably higher than those of River humic (Figure 4a, 5a, 7a-c, 8a-c), indicating a different dominant interacting mechanism. Also, these adhesion forces increased with increasing Na⁺ concentration (i.e., 0.29, 0.40, and 0.53 mN/m at 1, 10, and 100 mM Na⁺, respectively). These results suggest the influence of the concentration of monovalent ions in solution on the interacting mechanisms. Increasing concentration of Na⁺ ions in solution would reduce the repulsive electrostatic forces between citrate and ionized carboxyl groups on WW humic structure. Furthermore, Ca²⁺ ions had an impact on the adhesion behavior of WW humic to citrate-coated AgNPs. Both maximum adhesion forces and adhesion energies were higher than those in Na⁺ solutions (Figure 4b-5b, 9a-c, and 10a-c). A more efficient charge screening and also cation bridging mechanisms between Ca²⁺ and ionized carboxyl groups on both citrate and WW humic (i.e., dominant functional group on its structure) would cause this increased interaction. Nevertheless, this increase in adhesion forces and adhesion energies in Ca²⁺ solutions was not considerable compared to those in Na⁺ solutions. This result suggests that although cation bridging exerted an influence of WW humic adhesion to citrate, this mechanism was as dominant as in the previous experimental condition: River humic interactions with citrate-coated AgNPs in Ca²⁺ solutions.

Finally, force profiles between WW colloids and AgNPs-coated silica surface at 1 mM Na⁺ evidenced strong adhesion forces and long adhesion distances (>700 nm). Furthermore, multiple detachment events were also observed during interactions. Nevertheless, unlike the previous experimental conditions, the SEM micrographs obtained after experimentation showed multiple citrate-coated AgNPs adsorbed on the OM layer coating the AFM probe, indicating probe contamination (Figure S12). Therefore, the long adhesion distances, multiple

detachment events, as well as the strong adhesion forces observed would be explained by the detachment of the nanoparticles layer from the smooth silica wafer. This result indicates a high interaction between WW colloids and citrate even in monovalent cation solutions. These interactions would generate multiple strong adhesion forces that would cause detachment of nanoparticles from the iron-oxide coated silica wafer. Recent AFM studies investigating alginate/alginate interactions have reported large detachment distances during retracting, clearly indicating long-ranged interactions that exceed the expected length of the molecules of the adsorbed layer (i.e., likewise, WW colloids is enriched in polysaccharides and aminosugars).^{40, 41} Similar to the current study, these previous investigations suggest the formation of bridged long-chain organic molecules between the organic-coated AFM probe and the surface. This probe contamination was not detected during the previous experimental conditions using WW humic and River humic in Na⁺ or Ca²⁺ solutions, suggesting a different dominant interacting mechanism. As observed during the TR-DLS control experiments, hydrogen bonding played an important role during WW colloids interactions with citrate-coated AgNPs in solution. The aggregation of these nanoparticles was faster in WW colloids than in WW humic or River humic, and was clearly detected in even low ionic strength solution. Hydrogen bonding would be the dominant interacting mechanism also observed during AFM experiments that caused probe contamination (i.e., adsorption of nanoparticles on the WW colloids layer). Thus, calculations of maximum adhesion forces and adhesion energies were not conducted for this experimental condition. Remarkably, results from AFM and DLS consistently showed similar trends, thus providing strong evidence for the hypothesis of each interacting mechanism.

4. Conclusions: Environmental Implications

Silver nanoparticles (AgNPs) are nowadays one of the fastest growing classes of nanoparticles in industry. Nevertheless, the impact of these nanoparticles on the environment

or human health (i.e., bioaccumulation, toxicity, etc.) is not completely understood and is subject of high debate and research.⁴² Interestingly, a significant fraction of AgNPs (i.e., originally used in textiles, personal care products, or cleaning agents) is first released into wastewater.⁴ The current study suggests that the surface characteristics of AgNPs and the conditions of the media (i.e., ionic strength and presence of divalent cations) are fundamental to understand their behavior in a technosphere (e.g., wastewater treatment plant) and would influence their stability, aggregation behavior, transport, and interactions with other components such as effluent organic matter. Specifically, our results indicates that the functional groups of citrate (i.e., acting as capping agent) and the characteristics of the selected OM isolate (e.g., Hydrophilic organics of WW bio-colloid origin, hydrophobic humic-like structures of river/WW origin, etc.) play a fundamental role in controlling interactions in solution. In addition, the characteristics of these organics are highly dependent on their origins, and would be expected to exert a high influence on interactions with AgNPs. Consequently, OM associations with AgNPs would change the surface properties of these nanomaterials and affect their fate or stability on wastewater effluent, biosolids, or sewage sludge. This investigation would aid in providing a deep insight on the transport and fate of these nano-contaminants once discharged from urban wastewaters containing effluent organic matter (EfOM) into natural waters or agricultural fields. In addition, results from this study would also offer a head start for investigating AgNPs interactions with other hydrophilic organics of similar structure and hydrophilic character to WW colloids (e.g., biopolymers or membrane cell residues in biofilms).

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Associated Content

Supporting Information Available: aggregation kinetics of citrate-coated AgNPs under different solution conditions, probability density functions describing citrate-coated AgNPs maximum adhesion forces and adhesion energies to organic matter isolates, model retracting curves describing interactions between citrate-coated AgNPs and organic matter isolates, and SEM image depicting citrate-coated AgNPs adsorbed on WW colloids-coated colloidal probe during AFM experiments. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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List of Figures

Figure 1. Electrophoretic mobility of WW colloids, WW humic, and River humic organic isolates plotted against: a) Na^+ or b) Ca^{2+} concentration at unadjusted pH (5.8 ± 0.1)

Figure 2. Aggregation rate constants K_{11} of citrate-coated AgNPs measured at various monovalent or divalent cation concentrations and at unadjusted pH (5.8 ± 0.1).

Figure 3. Tapping mode images of: a) Smooth silica wafer, and b) Citrate-coated AgNPs adsorbed on a smooth silica wafer.

Figure 4. Mean values of maximum adhesion forces between citrate-coated AgNPs and River humic or WW humic in: a) Na⁺ solutions, and b) Ca²⁺-containing solutions.

Figure 5. Mean values of adhesion energies between citrate-coated AgNPs and River humic or WW humic in: a) Na⁺ solutions, and b) Ca²⁺-containing solutions.

TOC Graphic