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Investigating Initial Interactions Between Silver Nanoparticles and Wastewater

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Undergraduate Honors Thesis

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ADVISORY AND COMMITTEE SIGNATURE PAGE

This thesis has been approved by the Biological and Agricultural Engineering Department for submittal to the College of Engineering and Honors College at the University of Arkansas.


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1. Abstract

The use of nanoparticles (NPs) has increased exponentially in the last 15-20 years, especially in the consumer market. NPs are currently found in over 1800 commercial products, including cosmetics, clothing, packaging, and toys. As a result, NPs can enter the environment via wastewater (WW) streams, leading to new challenges in WW treatment. This study focuses on the initial fate of silver nanoparticles (AgNPs) in WW. The AgNP interaction including aggregation and dissolution in both synthetic and real WW were studied. Real WW was collected from the primary-clarifier, secondary-clarifier, and effluent WW streams at two local WW treatment plants (Westside and Noland) in Fayetteville, AR. In all cases, AgNPs had high rates of aggregation with salts and solids in real and synthetic WW (80.3%-99.8%). Of the non-aggregated AgNPs, there was no statistical difference in the concentration of Ag that passed through the nano (0.1 μm) and ionic (3 kDa) filters, indicating that either the AgNPs were small enough to pass through the ionic filter (<27 Ag atoms), or most of the non-aggregated Ag was present as ionic species rather than NPs. This merits further research.

2. Background

The use of NPs (NPs) for commercial and industrial applications has increased exponentially in recent years.¹ In fact, the Project on Emerging Nanotechnologies has already identified over 1800 nanoparticle-based consumer products from 622 companies in 32 countries.² The NPs can be suspended in gels and liquids, embedded in polymers, attached to surfaces, or used in industrial processing (like mechanical polishing fluids).³ Some popular applications are sunscreen,¹ food packaging, cosmetics,⁴ toothpaste, paints,⁵ medicines, coatings,⁶ antibacterial clothing,⁷ and even teddy bears.⁸

AgNPs, for instance, are among the most commonly used commercial metal/metal-oxide NPs [Me(O)NPs] because of their bactericidal properties.⁹ Of the nanoparticle-based products inventoried to date, at least 24% contain AgNPs.² Due to lack of regulation on this relatively new nanotechnology, many businesses withhold information regarding the quantities of NPs they are producing. It is estimated that the polyester fiber manufacturing industry alone produces 2.7-6.4 Mg of AgNPs, globally.¹⁰ Models estimate that the sunscreen industry produces 14.5-145 Mg of nano-TiO₂ (titanium oxide NPs) annually.¹¹ One study used limited company information along with phone surveys and proxy data to estimate production of nano-TiO₂, AgNPs, and nano-CeO₂ in the US.¹¹ Upper production bounds were modeled at 34,020, 18, and 635 Mg per year, respectively. This places production of nano-TiO₂ above production of trichloroethylene (18,960 Mg/year), the most common groundwater contaminant.¹²

The pathways for these NPs to enter the environment include construction, air pollution, and agrochemicals;¹³ yet the most prevalent pathway is through industrial and domestic WW. For example, one study demonstrated that an antibacterial exercise shirt can lose up to 2% of its NPs with just one rinse of water.⁹ NPs contained in one-time-use products will inevitably enter the environment, such as those in toothpaste, facial wash, sunscreen, cleaning supplies, or non-recyclable packaging. On the other hand, for products with longer “lifespans,” like paint, it is more difficult to predict NP release.¹⁴ Besides

product lifespan, NP pollution depends on chemical traits and interactions including particle size/distribution, crystal structure, surface charge, pH of the media, bulk/particle density, surface coatings, redox potential, porosity, and solubility.¹⁵

Emerging nanopollution is of significant interest to biological engineers, among many other disciplines, due to the cascading ecosystem and potential negative health effects. As nanotechnology has boomed in the last 15-20 years, there is a knowledge gap on long-term effects of NP exposure. However, short-term studies have shown that the same antimicrobial properties that make NPs useful in many products can negatively affect microorganisms in surface water.¹⁶ The particles are also defined by their high reactivity and tendency to aggregate,¹⁷ increasing the probability that they will bond with other pollutants (like cadmium and organics) and act as transporters for these pollutants throughout water, soil, and air.¹⁸ Researchers are being urged to focus on nanopollution treatment now, instead of waiting until the long-term consequences of exposure are realized. Preventative measures are only being taken seriously after disasters caused by asbestos, benzene, and chlorofluorocarbons.¹⁹

WW treatment plants (WWTPs) are key barriers between these potentially harmful pollutants and aquatic ecosystems, and therefore a primary area of work for biological engineers. Because nanopollution is a relatively recent phenomenon, it remains unclear how effectively it is being treated at certain WWTPs. Most facilities are not designed specifically to treat NPs, and complete removal has yet to be achieved.³ This is of increasing concern when WW is reused for drinking and irrigation purposes, like in Orange County, CA and Berlin, Germany.²⁰ Orange County faces worst-case concentrations of 147, 0.28, 0.037 $\mu\text{g/L}$ of nano-TiO₂, nano-ZnO, and AgNPs, respectively. Berlin has worst-case concentrations of 13, 0.25, and 3.3 $\mu\text{g/L}$ (nano-TiO₂, nano-ZnO, and AgNPs, respectively). Orange County uses their discharged WW to replenish the ground water, which becomes the source of drinking for the county. For this reason, advanced treatments (i.e. ultrafiltration and reverse osmosis) are necessary to mediate pollutants that would otherwise accumulate throughout this circular process. Berlin, on the other hand,

supplies their city's drinking water from aquifers that are replenished by local rivers and streams. The WW is discharged into these same rivers and streams, so there is also potential for circular transportation of pollutants. While concentrations this low currently remain unregulated, this is likely to change as more information is discovered about the fate of NPs in WW and the receiving streams, and places like Berlin and Orange County are taking preventative measures.

Various components in WW (e.g., solids, proteins, enzymes) can interact with AgNP and interrupt its fate and transport processes, directly impacting its removal within WW treatment. It has been shown that increasing the concentration of proteins in the water column decreases Ag^+ ion species via protein chelation (bonding) with released Ag^+ ions as well as coating AgNPs (thus preventing interactions with microorganisms).²¹ Sulfidation has also influenced AgNP speciation by reducing AgNP toxicity (which is attributed to Ag^+ ion).²² These studies showed that the NPs entering the WW stream may undergo transformation simply by interacting with WW constituents, which changes the resulting concentration in WWTPs. Thus, the objectives of the present research are to 1) synthesize and characterize AgNPs, 2) prepare WW samples from three sources (synthetic, and from two different WWTPs), and 3) combine AgNPs with WW and analyze concentration, aggregation, and dissolution of AgNPs. The result will help expose the true concentration of NPs in WW, subsequently impacting the selection of best removal strategy in WW treatment.

3. Methods

3.1 AgNP synthesis and characterization

The AgNP solution was created using a bottom-up synthesis technique by dissolving AgNO_3 to ionic form in water (solvent) and then converting these ions to NPs with sodium citrate or sodium borohydride as the reducing agent.^{23,24} AgNPs were characterized using ultraviolet-visible spectrophotometry (UV/Vis),²⁵ transmission electron microscopy (TEM), and inductively coupled plasma

mass spectrometry (ICP-MS) to identify the particle wavelength, diameters, and concentration in solution (respectively). UV/Vis (Beckman Coulter DU 720 Spectrophotometer, California) was used to analyze each sample at wavelengths between 300-1100 nm. Peaks for AgNPs were expected at 400 nm. The stock solution was stored in a brown bottle in the dark between uses.

AgNP stock samples were examined with TEM following a modified NIST protocol at the University of Arkansas' Nanoscale Material Science and Engineering Building's Materials Characterization Facility. NPs were fixed to copper TEM grids (Formvar/Carbon film; Electron Microscopy Sciences, Pennsylvania) by placing a droplet of AgNP solution on the sterile side of paraffin film (Parafilm M; Bemis Company Inc., Wisconsin), and then placing the grid on top of the droplet with forceps. The particles on TEM grids were viewed and photographed using the microscope (FEI Titan 80-300; Thermo Fisher Scientific, Massachusetts) with AMT camera (Advanced Microscopy Techniques, Massachusetts). Images of the NPs were analyzed using the ImageJ²⁶ software to determine nanoparticle counts and diameters.

3.2 WW preparation

Three different sources of WW were used in this experiment: synthetic WW and samples from two different WW treatment plants in Fayetteville, AR (Noland and Westside). Synthetic WW was produced using the recipe in Table 1. Synthetic WW recipe (1x concentration),²⁷ and then autoclaved. WW pH was adjusted to the recommended range of 7-8 by adding HCl or NaOH.

Table 1. Synthetic WW recipe (1x concentration)²⁷

Material	Conc., mg/l	Material	Conc., mg/l
Nutrient Broth	300	FeCl ₃ · 6H ₂ O	5
KH ₂ PO ₄	44	MnSO ₄ · H ₂ O	12.8
NaOH	25	(NH ₄) ₂ SO ₄	118.4
CaCl ₂ · 2H ₂ O	132.4	NaHCO ₃	467
MgSO ₄ · 7H ₂ O	100	KNO ₃	3
Glucose	140	NaCl	100

Real WW samples from both treatment plants were collected in 50 mL centrifuge tubes from the primary-clarifier (influent), secondary-clarifier, and effluent streams. Typical total suspended solids content of these three streams are 250 mg/L, <2.0 mg/L, and <0.1 mg/L, respectively.²⁸ Note that on the date of WW collection at the Westside treatment plant (March 30th, 2018) there had been significant preceding precipitation events. The plant normally processes 26.5 million L/day, but on the day of sampling the operators communicated that the plant processed an extra 49.2 million L of storm water.

3.3 Analysis of initial interactions

AgNP solution was added to synthetic and real WW samples in 10% volumetric combinations; 1 mL AgNP solution (about 1400 µg/L) was added to 9 mL of each WW sample. A 10% dilution was chosen because the peak at 400 nm in the UV/Vis scan was still visible, unlike with the 1% and 0.1% dilutions. Vortexing before every pipetting step was critical, because the NPs were suspended non-uniformly in solution, and error was significantly higher in preliminary experiments where frequent vortexing was not included. TEM was performed on samples of AgNP/WW solutions to visibly check for the presence of NPs and aggregation to solids in WW (see section 2.1) at magnifications of 50,000X-200,000X.

After adding 1400 µg/L of AgNPs to each WW solution, a series of filtrations was performed to measure concentrations of aggregated, nano, and ionic Ag with ICP-MS (iCAP TQ ICP-MS; Thermo Fisher, Massachusetts). The Ag detection limit was 1 µg/L. Sample preparation for ICP-MS requires a 2.5% concentration of nitric acid, therefore 0.357 mL of AgNP/WW solution was replaced with 0.357 mL of 70% nitric acid for every 10 mL of solution after filtration steps. The first non-filtered solution represented the “total Ag” concentration, or the concentration of added Ag.

To obtain the nano-sized Ag concentration, the AgNP/WW solution was filtered with a 0.1 µm filter (Acrodisc syringe filters; Life Sciences, Colorado), thus removing suspended solids in WW and the larger aggregates (>100 nm) formed between the AgNPs and WW (e.g., AgCl salts).

To find the ionic Ag concentration, acidified AgNP/WW solution was first filtered with the 0.1 μm filter, then placed in an ionic centrifugal filter (3kDa centrifugal membranes; Merck Millipore, Massachusetts) at 4714 rpm (4000 G) for 30 minutes. The difference between “total” or “added” Ag concentrations and the post-filtration Ag concentrations represent the quantity of Ag that aggregated/agglomerated with solids and/or other NPs.

Triplicates were measured for total, nano, and ionic Ag concentrations from each of the three WW streams for both treatment plants (54 samples total). With synthetic WW six replicates for total, nano, and ionic Ag concentrations were analyzed (18 total). Excel was used for statistical analyses of samples, namely analysis of variance (ANOVA) with least significant difference (LSD) separation of means. The confidence level was 95%.

4. Results & Discussion

4.1 AgNP characterization

The AgNP stock solution was successfully synthesized and contained a total Ag concentration of about 14 mg/L. The particles had an average nanoparticle diameter of about 11.0 ± 5.2 nm (Figure 1a; Table 4, appendix), which was comparable to the particles synthesized in the protocol article.²³ Engineered NPs have a diameter less than 100 nm in size, by definition.²⁹

However, for this study it was desired to generate NPs with diameters <30 nm because research has shown that particles at this size demonstrate shifts in crystallinity that modify environmental

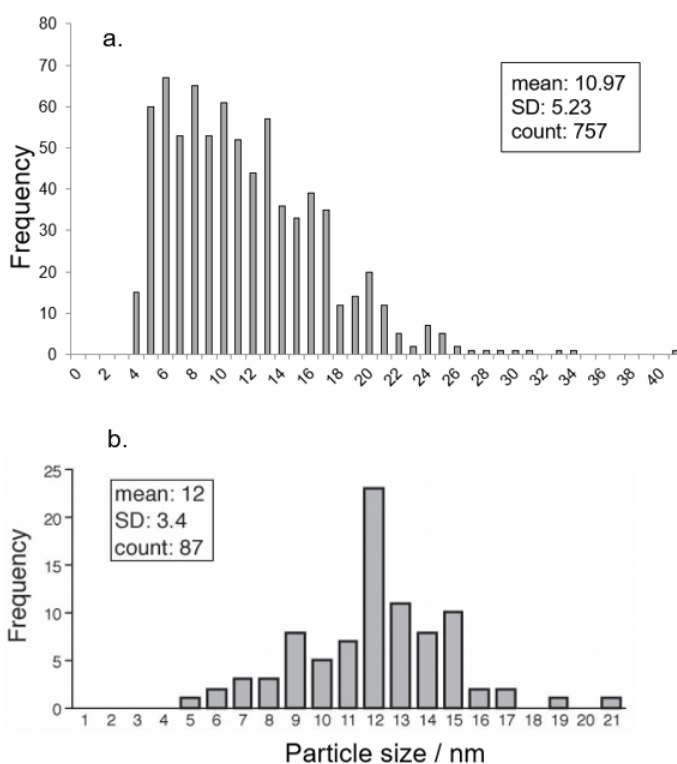


Figure 1. Particle size distributions of AgNP stock solution in the present research (a) and the reference for AgNP synthesis²³ (b).

reactivity.³⁰ The concentration of the stock solution was measured via ICP-MS twice (January and April 2018), and there was no statistical difference in concentration over the course of three months (t-test, $p=0.47$; Table 3, appendix), suggesting the particle stability of the AgNP stock solution. UV/Vis scans of the stock from July 2017 and January 2018 also verified that significant agglomeration of NPs had not occurred during storage (Figure 2). TEM allowed for visual confirmation of AgNP presence (Figure 3).

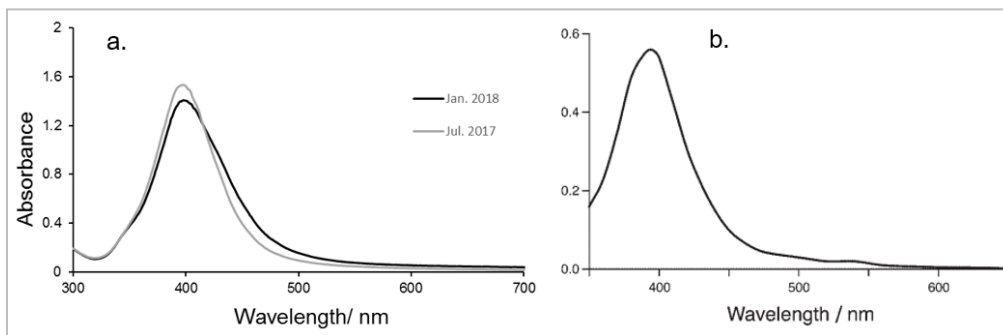


Figure 2. Comparison of UV/Vis scans of the AgNP stock solution in the present study (a) and from the reference source for AgNP synthesis²³ (b).

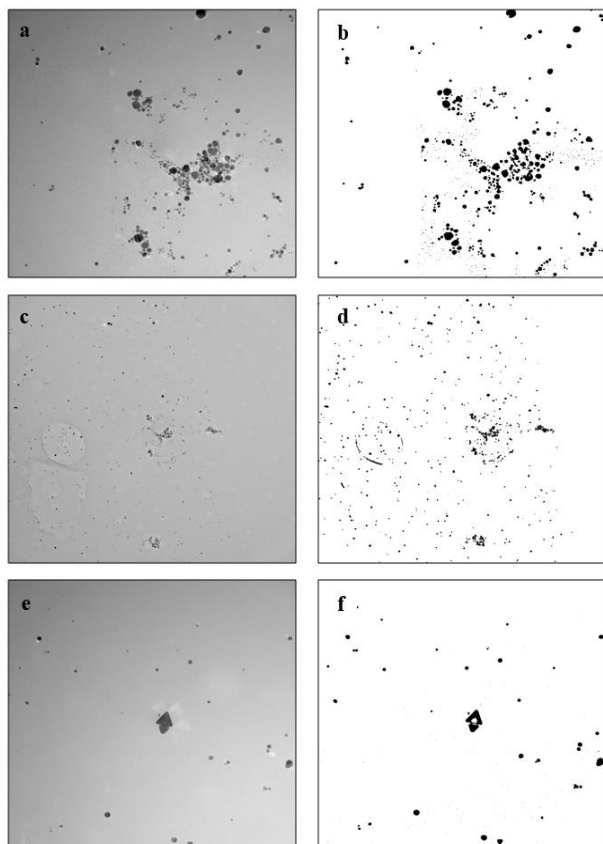


Figure 3 (right). Left column: TEM images of AgNP stock solution at 200,000X (a & e) and 50,000X (c). Right column: Images processed in ImageJ software to identify particles.

4.2 AgNP interaction with WW

The synthetic WW was made free of Ag; raw WW samples from Noland and Westside treatment plants were tested for the presence of Ag by using ICP-MS, and concentrations were less than the detection limit of $1 \mu\text{g/L}$, and thus negligible (Figure 4). Thus, to analyze AgNP fate about $1400 \mu\text{g/L}$ of AgNPs were added to the real WW samples, as well as the synthetic WW (10% solution volume). The exact amounts added to each sample were measured as “total Ag” with

ICP-MS and are presented in Figure 5. Even with frequent vortexing, there were statistical variations in the quantity of AgNPs added to each WW sample through volumetric dilution (ANOVA; $p=7.82 \times 10^{-8}$).

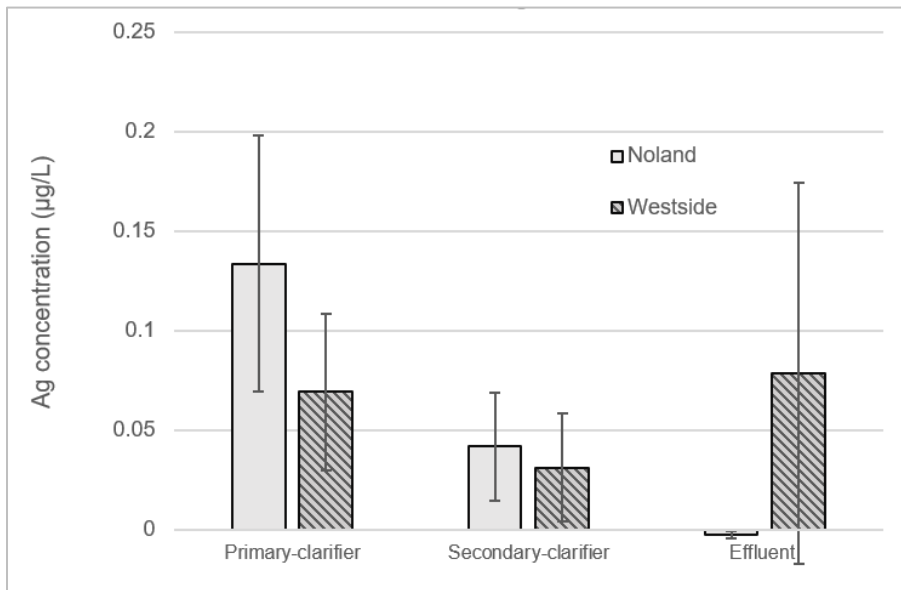


Figure 4. Negligible Ag concentrations in raw WW. ICP-MS Ag detection limit is 1 µg/L.

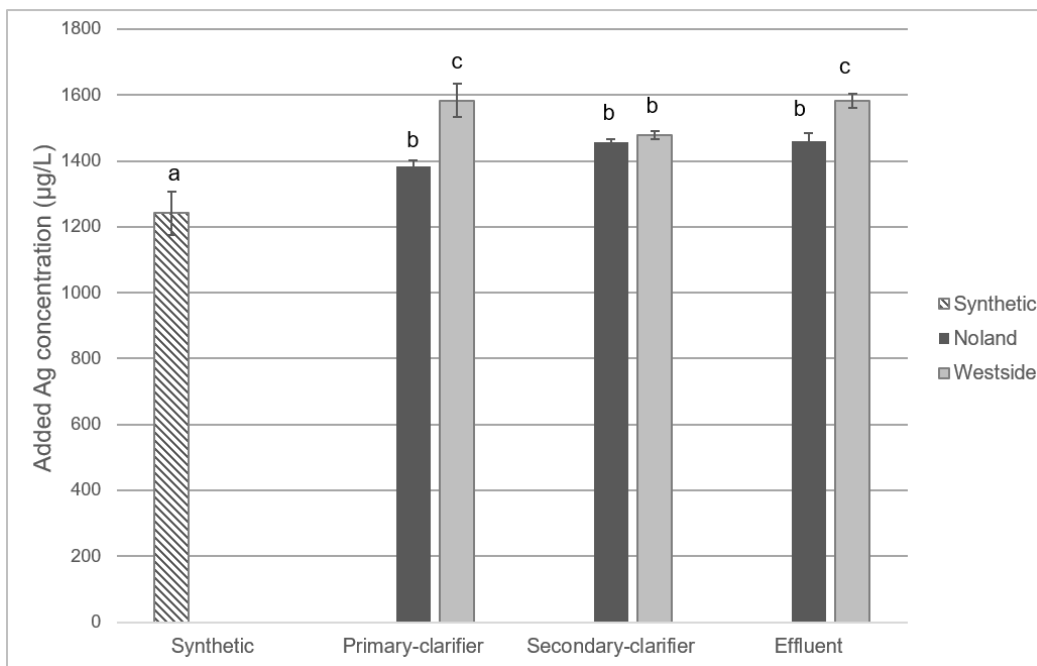


Figure 5. Total Ag added to each WW sample. Letters "a-c" denote statistical difference with ANOVA LSD; $p=7.82 \times 10^{-8}$.

TEM images revealed precipitates and clusters of AgNPs with salt crystals in the synthetic WW solutions (Figure 6). AgNPs are known to aggregate with salts; for example, one study found that in the presence of low NaCl levels (10 mM), AgNPs showed little aggregation, while at 100 mM NaCl all AgNPs were present as aggregates.³¹ Other studies support this complexation of AgNPs; for example, AgNPs are known to undergo chemical transformation in sewer networks by reacting with cysteine, histidine, sulfate, and chlorides.³²

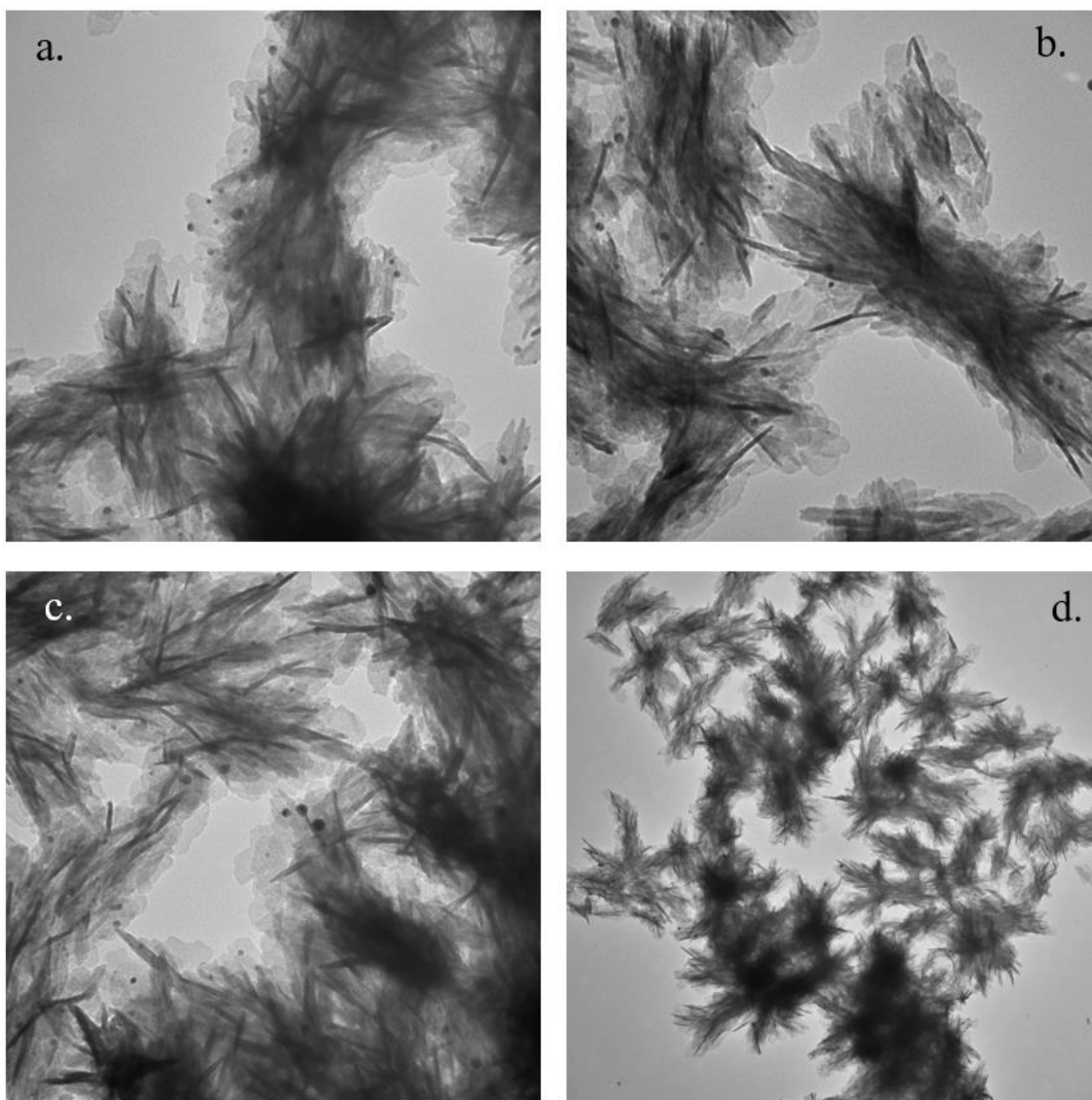


Figure 6. TEM images of AgNPs aggregated with salt crystals in synthetic WW; 200,000X (a, b, & c) and 50,000X (d) magnification.

The AgNP/WW solution was filtered through 0.1 μm filters to remove solids and the AgNPs that aggregated with the solids. After filtration with 0.1 μm filters, the solution was filtered through centrifugal filters to partition the non-aggregated Ag into “nano” and “ionic.” However, through ANOVA and LSD mean separations analysis it was determined that there were no significant differences between the concentration of nano and ionic Ag for each WW type (Figure 7), suggesting that the nano-sized Ag may have been small enough to pass through the centrifugal filter. The filter size was 3 kDa, which could pass AgNPs containing 27 or fewer Ag atoms (Ag molecular weight=107.86 Da/atom). Figure 8 is a condensed version of Figure 7, with averaged Ag concentrations for primary clarifier, secondary clarifier, and effluent streams from each WW source.

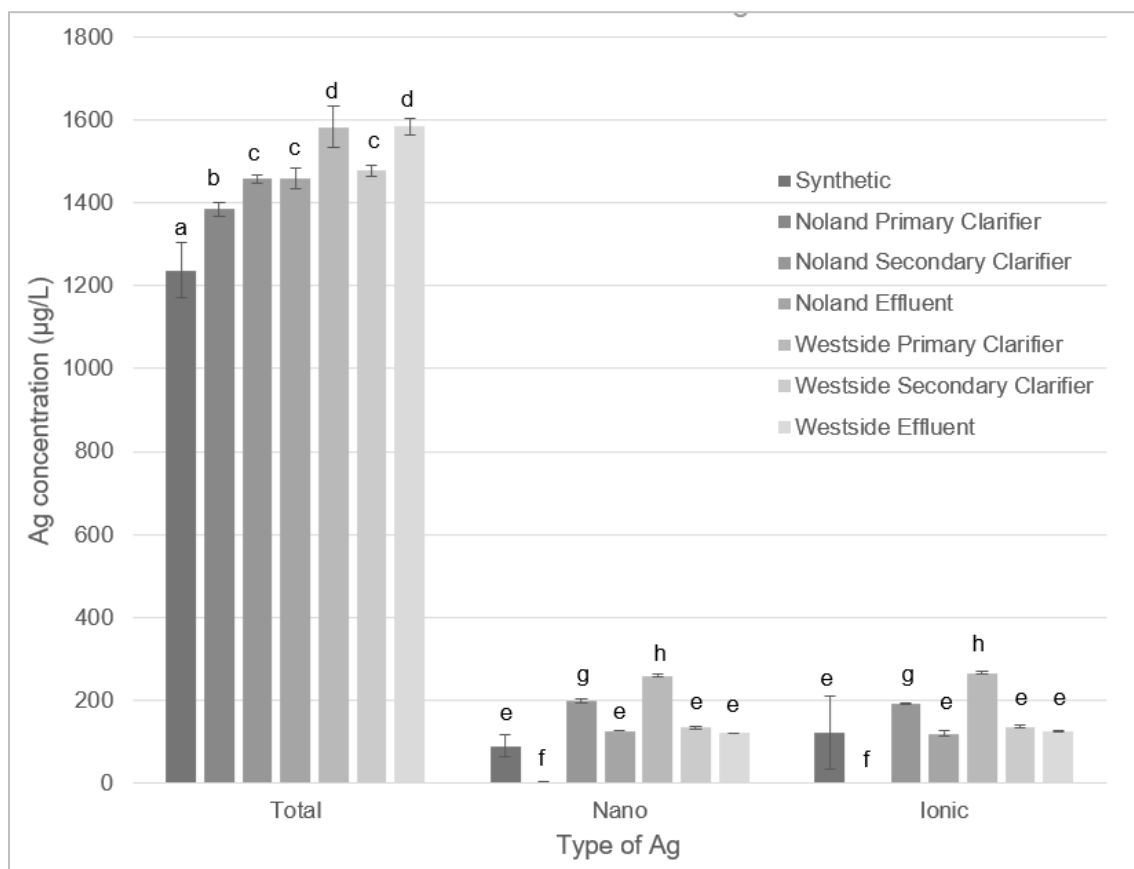


Figure 7. ICP-MS results for different types of Ag concentration in all wastewater sources. “a-h” denote statistical differences with ANOVA ($p=3 \times 10^{-56}$) and LSD for separation of means.

To find the concentration of aggregated Ag, the nano and ionic Ag concentrations were averaged (since there were no statistical differences) and subtracted from total Ag for each WW type (Figure 9). These data showed similar (and sometimes higher) percentages of aggregation (Table 2) than other studies that measured 70%-90% aggregation of AgNPs, depending on particle functionalization.³³

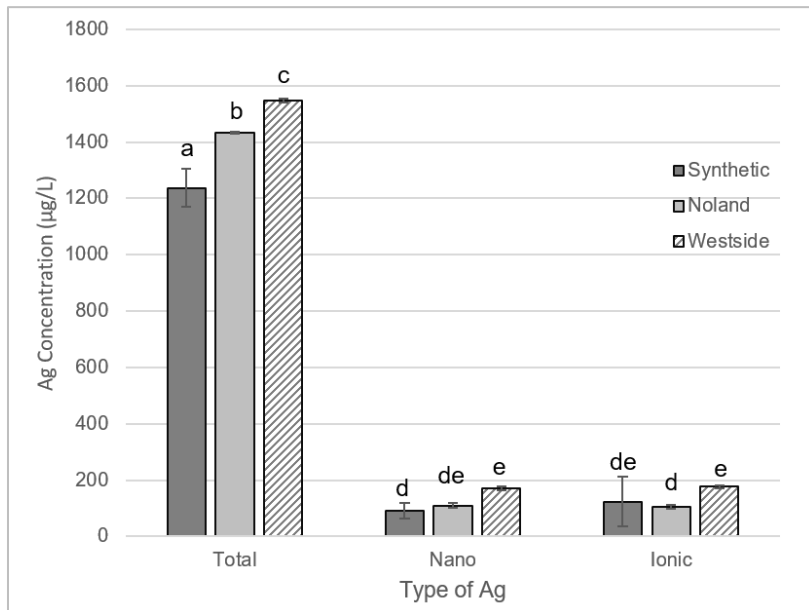


Figure 8. Fate of added Ag in WW samples from 3 different sources; concentrations from primary-clarifier, secondary-clarifier, and effluent streams from Westside and Noland were averaged.

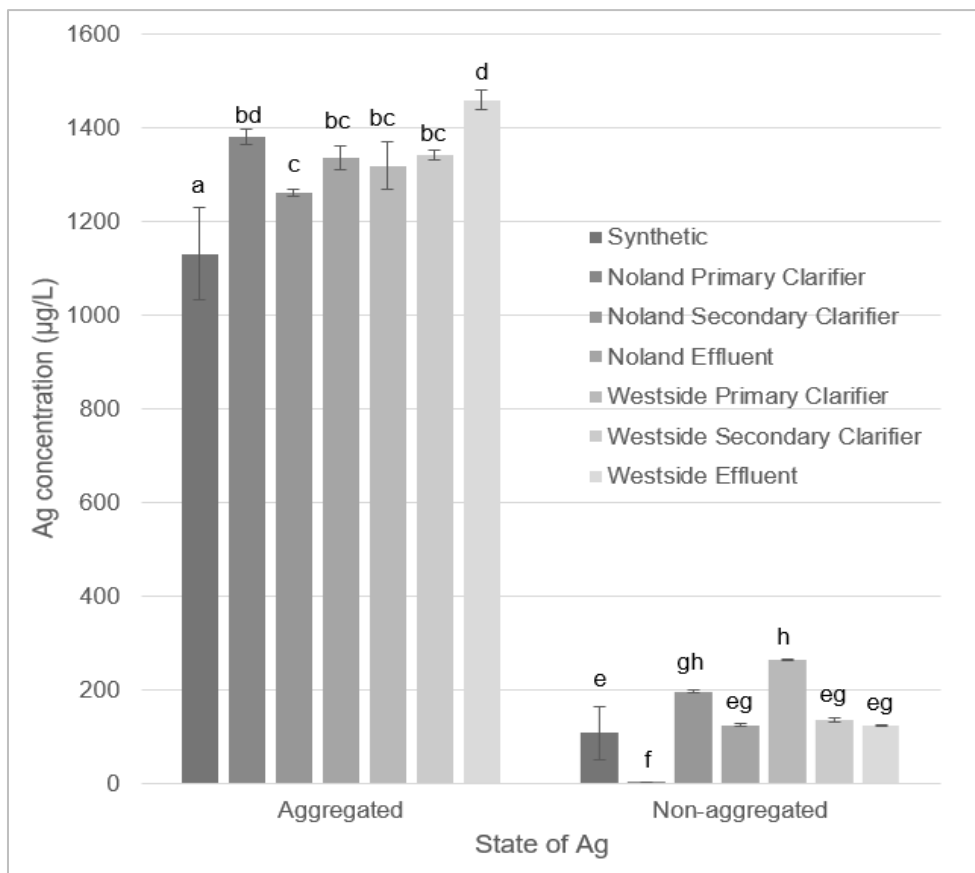


Figure 9. Concentrations of aggregated and non-aggregated AgNPs in WW samples.

Table 2. Percentages of Ag aggregation/non-aggregation in different WW samples.

	Synthetic	Noland			Westside		
		Primary-clarifier	Secondary-clarifier	Effluent	Primary-clarifier	Secondary-clarifier	Effluent
Aggregated	91.4%	99.8%	86.6%	91.5%	83.4%	90.8%	92.2%
Non-aggregated	8.6%	0.2%	13.4%	8.5%	16.6%	9.2%	7.8%

It was hypothesized that increased aggregation may be a function of greater WW solids content, and this was disproven. If it were the case, the primary-clarifier stream would show the most aggregation (250 mg/L of solids²⁸), followed by the secondary-clarifier (<2.0 mg/L), and effluent streams (<0.1 mg/L), which was not demonstrated in either plant. With Noland samples, aggregation was highest in the primary-clarifier stream, second-highest in the effluent, and lowest in the secondary-clarifier stream. With Westside samples, aggregation increased as solids content decreased (Table 2).

It is still uncertain whether the non-aggregated Ag remained as NPs suspended in solution or as ionic species. Studies suggest that AgNP dissolution into Ag⁺ ions is enhanced by the presence of oxygen and inhibited by chlorine.³⁴ This may contribute to aggregation trends measured in the Noland and Westside samples. At Noland, before the secondary-clarifier and effluent streams the WW is ozonated for disinfection (HyDOZ; BlueInGreen, Arkansas). Increased oxygen in these streams may have resulted in more AgNP dissolution and subsequently less aggregation in the secondary-clarifier and effluent streams as compared to the primary-clarifier stream. The secondary-clarifier stream, which showed the least aggregation (and possibly greatest dissolution), is closest to the ozonation system in that plant. However, this is speculation since dissolved oxygen measurements were not taken from any of the WW samples. This would be an insightful future research project.

5. Conclusion

The objectives of 1) synthesizing and characterizing AgNPs, 2) preparing WW samples, and 3) analyzing the initial interactions between AgNPs and WW were met. The majority of AgNPs added to both synthetic and real WW aggregated in solution (80.3%-99.8%) and were thus able to be filtered out with nano and centrifugal filters. Aggregation rates were slightly higher than what was found in the literature (70%-90%).³³ No difference was seen in the concentrations of AgNPs filtered by the 0.1 μm nano and 3kDa centrifugal filters, suggesting that the synthesized AgNPs were able to pass through the centrifugal filter, or that much of the Ag was present as ionic species. Therefore, non-aggregated AgNPs in WW solutions were not differentiated in this study. The results showed significant portion of Ag-NPs aggregate when entering the waste stream, suggesting the necessity of AgNP concentration measurement in real time. These findings contributed to understanding the composition of AgNPs in WW within Dr. Connie Walden's doctoral dissertation³⁵ on the *Fate of Silver NPs in Model WW Biofilms*.

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7. Appendix

Table 3. Statistical analysis of Ag concentration in AgNP stock between January and April.

t-Test: Two-Sample Assuming Equal Variances		
	AgNP stock, January ($\mu\text{g/L}$)	AgNP stock, April ($\mu\text{g/L}$)
Mean	14510.99	13680.15
Variance	2564259.14	762001.57
Observations	3	3
Pooled Variance	1663130.36	
Hypothesized Mean Difference	0	
df	4	
t Stat	0.79	
P(T<=t) one-tail	0.24	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.47	
t Critical two-tail	2.78	

Table 4. Statistical analysis of AgNP diameters from TEM images analyzed in ImageJ.

Diameter Statistics		
Mean	10.97	nm
Standard Error	0.19	nm
Median	10.11	nm
Mode	10.61	nm
Standard Deviation	5.23	nm
Sample Variance	27.35	nm
Kurtosis	2.06	nm
Skewness	1.10	nm
Range	37.19	nm
Minimum	3.62	nm
Maximum	40.81	nm
Sum	8305.65	nm
Count	757	

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