Citrate Stabilized Silver Nanoparticles: Study of Crystallography and Surface Properties

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ABSTRACT

Citrate stabilized silver (Ag) colloidal solution were synthesized and characterized for crystallographic and surface properties by using transmission electron microscopy (TEM) and zeta potential measurement techniques. TEM investigation depicted the size of Ag⁰ ranges from 5 to 50 nm with smaller particles having single crystal structure while larger particles with structural defects (such as multiply twinned, high coalescence and Moire patterns). ζ-potential measurement confirms the presence of Ag⁺ in nAg stock solution. The shift in ζ-potential measurement by +25.1 mV in the filtered solution suggests the presence of Ag⁺ in Ag⁺ nanoparticles.

Keywords: Agglomeration, Citrate Stabilized, Coalescence, Crystallography Study, Multiply Twinned, Silver Ions, Silver Nanoparticles, Transmission Electron Microscopy (TEM), Zeta Potential

1. INTRODUCTION

The application of nanoscale materials and structures, usually ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion (Atwater & Polman, 2010; Brown et al., 2011), catalysis (Bhattarai, Casillas, Ponce,
& Jose-Yacaman, 2012; Cuenya, 2010; Khanal, Casillas, Velazquez-Salazar, Ponce, & Jose-Yacaman, 2012; Raji, Chakraborty, & Parikh, 2012; Yuan, Yan, & Dyson, 2012), medicine (Conde, Doria, & Baptista, 2012; Davis et al., 2010), and water treatment (Dankovich & Gray, 2011; Kaegi et al., 2011). In recent years, noble metal nanoparticles like gold (Au), silver (Ag) etc. are of special interest due to their plasmonics properties, especially in photovoltaic (Pudasaini & Ayon, 2012; Tan, Santbergen, Smets, & Zeman, 2012), medicine (Conde et al., 2012; Davis et al., 2010; Nykypanchuk, Maye, van der Lelie, & Gang, 2008), and bio-imaging (Hutter & Maysinger, 2011; Lee et al., 2006; Y. Liu, Miyoshi, & Nakamura, 2007). Silver nanoparticles are extraordinarily efficient in absorbing and scattering light and, unlike many dyes and pigments, have a color that depends on the size and the shape of the nanostructures.

The interest in Ag nanoparticles and their applications has increased mainly due to their important antimicrobial, antifungal, antibacterial, antiviral activities (Jung et al., 2008; J. Liu, Yu, Yin, & Chao, 2012), allowing their use in several medical applications. Colloidal silver is of particular interest given its distinctive properties, such as good conductivity, chemical stability, catalytic and enhanced antibacterial activity. There is an increasing interest in understanding the relationship between the physical and chemical properties of nano silver and their potential risk to the environment and human health. The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulphydryl) groups (Toshima et al., 1991), although other target sites remain a possibility. Amino acids, such as cysteine, and other compounds containing thiol groups, such as sodium thioglycolate, neutralized the activity of silver against bacteria (Liau, Read, Pugh, Furr, & Russell, 1997). On the other hand, disulfide bond-containing amino acids, non-sulfur-containing amino acids, and sulfur-containing compounds, such as cystathione, cysteic acid, L-methionine, taurine, sodium bisulfates, and sodium thiosulfate, were all unable to neutralize the activity of silver ions. These and other findings imply that the interaction of silver ions with thiol groups in enzymes and proteins play an essential role in its antimicrobial action, although other cellular components, like hydrogen bonding, may also be involved.

Nonetheless, colloidal Ag has been utilized for centuries but only recently it has gained notoriety; most famously as a drinkable solution marketed by alternative medicine practitioners who herald it as a “cure-all”. Despite this pseudo-science, real research is underway concerning colloidal Ag solutions because the mechanism of microbial cytotoxicity is not fully known. Moreover, the properties of specific nanostructures that will optimize microbial cytotoxicity are not, as of yet, defined because the properties of Ag nanostructures themselves are not fully understood. At present, the adverse effects of Ag NPs on wastewater treatment and the environment is not completely known. However, free silver ion (Ag\(^+\)) is highly toxic to a wide variety of organisms including bacteria. To this end, understanding the properties of silver nanoparticles and silver ions is very important to effectively control its activity. In order to control the nAg and silver ion activity, we need to understand, how the size, morphology, pH, surface coating, solution chemistry, crystalline nature of nanoparticles and surface charge affect the ion release mechanism.

The mechanism of toxic properties of Ag NPs has not been clearly elucidated. It is generally believed that the toxicity of Ag NPs is related to release of Ag\(^+\) ions from the Ag colloidal solution (Chao et al., 2011; Kennedy et al., 2012). However, the effects are not due simply the release of Ag\(^+\) into the surrounding environment, as the Ag NPs effects are distinct from those of Ag\(^+\) alone and depend on Ag NPs size and coating (G. A. Sotiriou, A. Meyer, J. T. N. Knijnenburg, S. Panke, & S. E. Pratsinis, 2012). To assess the risk of exposure and further understand the Ag NP effects, information on
the concentration, size, and form (aggregates, agglomerates) of Ag NPs, as well as the Ag\(^+\) concentration should be investigated. Given the release of Ag\(^+\) from Ag NPs and the transformation of Ag\(^+\) into Ag NPs in the environment, Ag\(^+\) and Ag NPs commonly coexist and it is of great importance to develop methods for specific analysis of AgNPs and Ag\(^+\). Several studies have been conducted in which the size of the silver nanoparticle had an effect in the amount of ions released (J.R. Morones et al., 2005; G. A. Sotiriou, A. Meyer, J. T. Knijnenburg, S. Panke, & S. E. Pratsinis, 2012). Another study showed that by changing the pH of the solution, the silver ion release was enhanced (Sotiriou et al., 2012). Nonetheless, further research on the effect of crystallinity of the nanoparticles on the ion release mechanism is lacking.

In this respect, a better understanding of the shape and size of Ag nanoparticles and its insight investigation of crystalline structure is very crucial. The fundamental properties like, chemical, biological, medicinal, plasmonics, catalysis are inherently related with its atomic structure. By suitable choice of nucleation and growth kinetics along with the use of suitable surfactant, the shape of Ag nanoparticles can be precisely controlled. Recently, Li et al. synthesized citrate stabilized quasi spherical Ag nanoparticles with well defined morphologies by the synergistic effect of ascorbic acid, sodium citrate and potassium iodide (Li, Xia, Wang, & Tao, 2013). Significant studies have been done in controlling the nanoparticle shape, size and the final structure, which can be achieved by controlling the crystalline structure of the seed and the rate of addition of atoms in the seed mediated growth process (Wiley, Sun, Mayers, & Xia, 2005; Wiley, Sun, & Xia, 2007; Xia, Xiong, Lim, & Skrabalak, 2009; Bhattarai, Casillas, Khanal, Velazquez Salazar, Ponce & Yacaman, 2013). Nonetheless, in a single step process, the nucleation and growth take place simultaneously, controlling the shape will be complicated, which is determined by various factors such as surfactants, reduction kinetics, oxidative etching etc. In this paper, we report the characteristic properties of citrate stabilized Ag nanoparticles, its surface and crystallographic properties. The shape, size and crystalline structure of nanoparticles were investigated using transmission electron microscopy (TEM) and briefly discuss about the formation of larger nanostructures. Moreover, the surface properties of Ag nanoparticles are investigated using the ζ-potential measurement technique. The shift in ζ-potential measurement by +25.1 mV in the filtered solution is the evidence for the presence of Ag\(^+\) in Ag\(^o\) nanoparticles.

2. EXPERIMENTAL METHODS

2.1. Chemicals

All the chemicals sodium borohydride (NaBH\(_4\)), trisodium citrate (Na\(_3\)C\(_6\)H\(_5\)O\(_7\)), silver perchlorate (AgClO\(_4\)) were obtained from Sigma-Aldrich and used as received without further purification. Deionized water was used throughout the whole experiment.

2.2. Synthesis of Ag Nanoparticles

Ag colloids were synthesized by reducing AgClO\(_4\) with NaBH\(_4\) in the presence of Na\(_3\)C\(_6\)H\(_5\)O\(_7\), as the stabilizer (J. Liu & Hurt, 2010). NaBH\(_4\) (2 mM, 0.0044 gm) and Na\(_3\)C\(_6\)H\(_5\)O\(_7\) (0.6 mM, 0.01044 gm) were measured and mixed with deionized water (59.2 mL) and vigorously stirred in ice bath for an hour. Then, 0.0025 gm of AgClO\(_4\) (15 mM) dissolved in 0.8 mL water was added to above solution. As it was added, the yellow color is obtained indicating the formation of Ag nanoparticles. The reaction was allowed to complete three more hours and the soluble byproducts were removed by centrifugation at 6000 rpm for 10 minutes. Finally, the TEM sample was prepared by placing 3-4 drops of colloidal nanoparticles dispersed in water on a holy carbon film coated Cu grid (3 mm, 300 meshes) and dried at room temperature.
2.3. Changes of pH

The pH of the solution was changed from 7.52 to 3.25 by adding 1 M HCl and the corresponding ζ-potential was recorded.

2.4. Characterization

The obtained Ag NPs were characterized by using UV-Vis absorption spectroscopy, ζ-potential and transmission electron microscopy (TEM). The absorption spectra were measured in 300 – 600 nm range by using a UV-Vis spectrophotometer (Cary, Model 14R). The ζ-potential was recorded by using Zetaseizer. The concentration of nAg particles (Ag⁰, Ag⁺ and Ag⁰/Ag⁺) were determined by atomic absorption (AA) spectroscopy. The shape, size and crystalline structure of the nanoparticles were studied using transmission electron microscopy (TEM) JEOL JEM-2010F operated at 200 kV with a 0.19 nm point resolution. The solution was then filtered to remove Ag⁰ by centrifugal ultra filtration, 2 times at 5000 rpm for 10 minutes, and ζ-potential was recorded for both cases.

3. RESULTS AND DISCUSSIONS

3.1. TEM Characterization

The shape, size and crystalline structure of Ag nanoparticles were studied using TEM operating at 200 kV. Figure 1(a-c) shows the low magnification TEM images of obtained Ag NPs that contains all three species (Ag⁰, Ag⁺ and Ag⁰/Ag⁺). There is very wide distribution in size ranging from 5 nm to 50 nm as represented by histogram inset in Figure 1(a). More than 50% of particles have size less than 10 nm while the other structures are larger in size (10 to 50 nm). The shape of the particle is not well defined but smaller particles have a spherical like shape as compared to larger structures with a variety of

Figure 1. (a-c) Low magnification TEM images of as synthesized Ag nanostructures. There is wide range of size from 5 to 50 nm. The inset in Figure 1(a) is the histogram of size distribution and is presented for 100 particles and shows that more than 50% of particles are <10 nm in size and others are larger. The larger particles are formed from the coalescence of smaller particles.
shapes. In a statistical study, for particles of 1 to 10 nm in size, about 98% of these particles were found to be octahedral (single crystal) and larger particles were found to be icosahedral or decahedral in shape with multiply twinned structures.

Figure 2(a-c) represents the TEM micrograph of smaller nanostructure. Those structures are less than 10 nm with single crystal nature. As time passes, those small particles get coalesced with other particles and larger structures are formed. The HRTEM images of some multiply twinned and super crystal structure is presented in Figure 2(d-g). In the case of small particles, the cyclic twinning suggesting a five-fold symmetry composes a circular stacking of twinned tetrahedral units forming a decahedron. Theoretical calculations have shown that the formation of decahedral particles are more favorable (Elechiguerra, Reyes-Gasga, & Yacaman, 2006). These decahedrons have a total free energy lower than that of the Wulff polyhedron (Xia, Xiong, Lim, & Skrabalak, 2009). As the multiply twinned seed increases in size, the strain energy caused by the twin defects increases as well. We observe some Moiré patterns in the structure that might be the result from superposition of two crystals. In the case of silver, with low stacking fault energy (20-30 mJ m$^{-2}$) (Dahmen et al., 2002), twins as well as stacking faults are readily formed during crystal growth (Elechiguerra et al., 2006).

Figure 2. Some representative TEM images of smaller (~10 nm) and larger (30-45 nm) sized nanoparticles. (a-c) HRTEM images of smaller (5-10 nm) sized Ag nanoparticles. (d-g) TEM images of larger spherical particles produced with the size around 45 nm. Some of the smaller particles are single crystals while others are twinned. Twinning and Moiré pattern fringes are observed in the larger structures.
both the strong and weak spot and the obtained structure is presented in Figures 3(d) and 3(e). Figure 3(d) gives the contribution from the mask applied in the strong spot and Figure 3(e) from weak spot. There is some contribution from the weak spot there by confirming the super lattice structure. In some cases we observe the Moire patterns as well.

Figure 4 represents the unusual crystal structure obtained during TEM observation. The FFT corresponding to region R1 and R2 are presented in Figures 4(b) and 4(c), and it shows that the region R1 is made from the superposition of the lattices and region R2 is free from this. Figure 4(d) represents the magnified image of region A showing the junction between two lattices. This is an example of stacking faults in different planes, which commonly result in partial dislocations (Elechiguerra et al., 2006).

As it was mentioned before about the coalescence of particles, the actual coalescence from TEM micrograph is presented in Figure 5(a-d). We can see the coalescence between different sized particles during their formation. Figure 5(a) represents the combination between two crystals, forming the new crystal with the formation of neck thereby stabilizing the new particles. The inset in Figure 5(a) represents the neck formation by bridging two crystals. Two different lattice structures are clearly seen in two sides of the structure. Figure 5(b) represents the coalescence between a small and
large particles. Figures 5(c) and 5(d) represent the coalescence of three and more particles. In our study, the larger structures are obtained from the superposition and coalescence between different particles. In a study by Polte et al., it was reported that the in situ synthesis of silver nanoparticles via the reduction of silver perchlorate using sodium borohydride requires a nanoparticle growth of small particle (2-3 nm) aggregation (Polte et al., 2012; Van Hyning & Zukoski, 1998). This mechanism is based on a stabilization of small Ag nanoparticles by BH$_4^-$ for a very short period of time. According to Polte et al., the growth of Ag nanoparticles with NaBH$_4$ as the reducing agent is mainly due to the coalescence even after a stabilizing agent has been added. Given that NaBH$_4$ is a strong reducing agent, the reduction is faster than the particle growth; hence the lack of particle stability leads to aggregation, which is needed for the coalescence process.

As previously explained above the Ag$^+$ ions were obtained by filtering the stock solution. The filtered solution was examined in the TEM and we were unable to see the particles. However, in some cases we observed very few particles (single crystals and twinned) (See Figure 6). Those nanostructures obtained from the coalescence of Ag$^+$ ions.

### 3.2. Optical and Chemical Characterization

The UV-Vis spectrum of the obtained nanostructure is presented in Figure 7 where the Ag Plasmon absorption peak is clearly seen at 412 nm. This peak has a higher absorbance, but is similar in shape and near to the 430 nm peak obtained by Liu, et al. (F.-K. Liu, Ko, Huang, Wu, & Chu, 2005), that was indicative of their spherically shaped Ag$. The obtained nanostructure was further characterized using AA spectroscopy and the concentration was found to be 6.64 mg/L.

It has already been reported that Ag nanoparticles release Ag$^+$ ions. In order to investigate the presence of Ag$^+$ ions, the ζ-potential measurement was carried out. The ζ-potential gives the surface charge distributions of all the particles and charges present in the synthesis. In a separate experiment, the pH of the solution was changed from 7.52 to 3.25 and the
corresponding ζ-potentials were measured. The ζ-potentials were found to be -46 mV, -43.40 mV, -19.40 mV, -11.90 mV and 0.292 mV for pH 7.52, 6.64, 5.22, 4.85 and 3.25 respectively (Figure 8). It is observed that the ζ-potential was increased with the decrease in pH. The H⁺ resulted from the addition of aliquots of 1M hydrochloric acid (HCl) increasing the ζ-potential. We measured the ζ-potential before and after the filtration of Ag colloids (see the inset of Figure 8). The ζ-potential of the synthesized nanoparticles (nAg, Ag°, Ag°/Ag⁺) was found to be -46 mV (left curve in the inset) and -20.9 mV after filtration (right curve in the inset). This indicates the increase in ζ-potential after filtration due to the increase in Ag⁺ concentration after filtration. The measured ζ-potential of Ag° was comparable (Feng et al., 2000) to at -46 mV, and suggests stabilization by a surface layer of citrate anions (Jose Ruben Morones et al., 2005). The ζ-potential shifted to -20.9 mV indicating, as expected, the increase of surface charge in the filtrate now dominated by Ag⁺. The change in ζ-potential of the unfiltered (stock) solution under change in pH, was studied and it was determined that the solution behaves as expected. By LeChatelier’s Principle, adding an excess of H⁺ to the equilibrium state of the stock solution, the reaction will proceed to the right, forming an excess of Ag⁺: 2Ag +1/2O₂ + 2H⁺ ↔ 2Ag⁺ + H₂O.
Figure 6. HRTEM images of smaller sized particles after filtration. There should be no particles after filtration; however, those particles might be produced from the coalescence of ions as time passes.

Figure 7. UV-Vis absorption spectrum of as obtained Ag nanoparticles in water. The absorption peak was found at 407 nm.
4. DISCUSSION AND CONCLUSION

The size of the synthesized silver nanoparticles depends on several factors including, stabilizing agent, coalescence process, nucleation, thermodynamics and particle growth kinetics. The synergetic combination of several factors leads to the formation of stable nanoparticles. During the reduction of $\text{AgClO}_4$ with $\text{NaBH}_4$, agglomeration of small particles takes place thereby forming larger nanostructures. The shape of Ag NPs is highly size dependent. Smaller particles (1-10 nm) are more likely to favor octahedral single crystal shape and larger particles (>20 nm) are more likely to result in multiply twinned shapes with icosahedral and decahedral structure. TEM images show particles with size distribution ranging from 5 to 50 nm with more than 50% of particles having a size less than 10 nm.

The TEM images revealed the coalescence of small nanoparticles forming larger nanostructures with wider size distribution. This can be attributed due to the higher Ag$^+$ concentration in the solution. The coalescence process is due to the reduction of $\text{AgClO}_4$ with, a strong reducing agent ($\text{NaBH}_4$), which is one of the causes for particle instability. The coalescence of nanoparticles produced larger multiply twinned...
structure thereby inducing structural defects (stacking faults, partial dislocation, etc.) and ultimately changing the surface energy of the particles. These structure defects is a likely cause for the observed Moire pattern revealed in the TEM image.

A solution of silver nanoparticles was prepared to characterize and prove the existence of three species: Ag⁺ ions, Ag° and Ag⁺ ions adsorbed on Ag°. The solution was synthesized using a traditional method. The added citrate in Ag nanoparticles colloids, serving as a stabilizer, binds strongly to the Ag⁺ and facilitates the reduction and subsequent capping of Ag°. The ζ-potential measurements before and after filtration of the Ag colloids, depict the shift in potential from -46.0 mV to -20.9 mV. The strong ionic presence in the solution after filtration is responsible for the peak shift. Furthermore, the change in pH of the Ag colloids has immense effect on the observed ζ-potentials shift. We observed by increasing the pH, the ζ-potential peak shifts to the right. In order to better understand the toxicity and antimicrobial effect of silver nanoparticles, it is important to know what does a silver nanoparticle solution contain and how does each species contribute to the overall toxicity. Once, the contribution of each species is known, they can be controlled to effectively induce its toxicity. Since the Ag nanoparticles containing silver ions have proven to be more effective, this study will help to gain some insights about producing and controlling these ions.

In summary, we are able to prove the existence of Ag° in Ag colloids using TEM images. Several structural defects, such as dislocations, twinning and Moire patterns were observed, which suggest that the high Ag⁺ concentration leads to defects or superposition of lattices. The larger multiply twinned structures are introduced from the twin boundary defects in order to minimize the surface energy. We observe a shift in the ζ-potential due to an increase of ionic concentration (Ag⁺). This surface properties and crystallographic information will be helpful for future applications of citrate stabilized Ag nanoparticles.

ACKNOWLEDGMENT

The authors would like to thank Dr. Miguel Jose-Yacaman for his guidance in this project and funding for the experiment. We would also like to thank Dr. Kelly Nash and Yasmin for their help in ζ-potential measurements. The funding from Physics and Astronomy Department is appreciated.

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