

# Synthesis and Characterization of Silver Nanoparticles Anchored on Montmorillonite via Chemical Reduction

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**Abstract.** Montmorillonite clay particles were decorated with silver (Ag) nanoparticles by chemical reduction of Ag nitrate with sodium citrate. X-ray diffraction (XRD) and energy dispersive X-ray spectrometry (EDS) confirmed the presence of metallic Ag on the surface of montmorillonite. The average crystallite size of the Ag nanoparticles obtained from the broadening of the 111 Ag peak ranged at 13-16 nm. On the other hand, the apparent particle sizes obtained from the SEM images were about 79-128 nm, suggesting that the nanoparticles are polycrystalline and possibly agglomerated. The increase in the concentration of reducing agent produced smaller Ag nanoparticles with narrower size distribution. The antibacterial test showed that the Ag nanoparticles, with mean size of 79 nm, adsorbed on montmorillonite were able to inhibit the growth of *Staphylococcus aureus* (*S. aureus*) with an antimicrobial index of 0.4.

**Index Terms:** Antibacterial, Bacteria, Chemical Reduction, Montmorillonite, Nanoparticles, Nanosilver, Silver

## 1 Introduction

In recent years, there have been alarming outbreaks of infectious pathogens which are a significant burden on global economies and public health. Growth of population along with urbanization with poor water supply and environmental hygiene has allowed for the transmission of infectious pathogens to the communities. These have caused outbreaks of diseases such as influenza (A/H5N1), diarrhea (*Escherichia coli*), and cholera (*Vibrio cholera*) throughout the world. Comprehensive treatment of environments containing infectious pathogens using disinfectant nanomaterials were proposed to prevent such outbreaks.<sup>[1]</sup>

Nanoparticles are defined as small particles with sizes ranging from 1 to 100 nm.<sup>[2]</sup> These nanomaterials are of great interests in the industry and academe due to their interesting and diverse properties which deviate from their bulk counterparts.<sup>[3]</sup> Metallic nanoparticles have size and shape-dependent properties for applications such as catalysis, optics, and data storage. Silver nanoparticles (Ag NPs) in particular have distinct physio-chemical properties, high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability and catalytic activity.<sup>[4]</sup> They have been widely used commercially in clothing, food industry, paint, sunscreens, cosmetics and medical

devices.<sup>[5]</sup> They have also been used in bio sensing, water purification treatments, and drug delivery technologies due to their antimicrobial properties.<sup>[6]</sup>

Particularly, higher antibacterial activity of Ag NPs has been exhibited by smaller Ag NPs due to their surface area. The decrease of size to nanoscale increases the surface area-to-volume ratio hence increasing its reactivity to its surroundings as more amount of atoms is available for interaction.<sup>[7]</sup> The factors affecting its bactericidal effect are size, shape, size distribution, morphology, surface functionalization, and stability.<sup>[8]</sup>

In general, Ag NPs kill bacteria through damaging the cell wall, triggering the formation of excessive free radicals, and reacting with major cell components.<sup>[8-11]</sup> Smaller particle size allows the particle to reach the nucleus of the bacteria and create broad contact to interfere with the effectiveness of enzymes. Consequently, this slowly kills metabolic responses.

The synthesis of Ag NPs can be done by physical methods, such as evaporation-condensation<sup>[12]</sup>, ceramic heating<sup>[13]</sup>, arc discharge<sup>[14]</sup>, and laser ablation<sup>[15]</sup>. Another approach is through chemical reduction wherein the Ag NPs are produced by reduction of Ag ions to Ag(0). This method has three components: Ag precursor, reducing agent and the stabilizing agent. Common reducing agents include trisodium citrate<sup>[16]</sup>, borohydride<sup>[17]</sup>, ethylene glycol<sup>[18]</sup>, formaldehyde<sup>[19]</sup>, ascorbic acid<sup>[20]</sup>, tollens reagent<sup>[21]</sup>, and hydrazine hydrate<sup>[22]</sup>. However, stabilization of Ag NPs poses a problem as they agglomerate and without a carrier, readily surge and attack microbes at once, which is not ideal for time related applications.<sup>[17]</sup> Thus, it is important to study how to control

the size of the Ag NPs, as well as provide a stable carrier for the nanoparticles to impede mass diffusion.

In this study, Ag nanoparticles were synthesized via chemical reduction and anchored on montmorillonite (MMT) surface to serve as carrier of nanoparticles. The effect of reducing agent concentration, in this case trisodium citrate, on the particle size of Ag nanoparticles was investigated. The antimicrobial activity of the synthesized Ag nanoparticles against gram positive *Staphylococcus aureus* (*S. aureus*) and gram negative *Escherichia coli* (*E. coli*) was also determined by agar diffusion test.

## 2 Methodology

### Anchoring of Ag Nanoparticles on Na<sup>+</sup>-MMT

First, 0.394 g silver nitrate (AgNO<sub>3</sub>, Laboratory grade Merck Millipore) was dissolved in 116 mL of distilled water. The flasks were covered with aluminum foil to avoid photoreduction of Ag(I) ions. Then, 2.5 g of Na-rich montmorillonite organoclay (Na<sup>+</sup>-MMT, Materials Science Division – Industrial Technology Development Institute of the Department of Science and Technology) powder was added into the Ag(I) aqueous solution with stirring at 1150 rpm for 12 h at room temperature. The concentration of Ag(I) in solution is about 0.02 M. Then, 11.6 mL 0.20 M trisodium citrate (Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>, RTC Laboratory Services and Supply House) was added dropwise into the Ag(I) solution to form the Ag nanoparticles. The ratio of Ag(I) to citrate ranges from 1 to 2. The Ag<sup>+</sup>/MMT solution was heated to 100°C with stirring. The reaction was allowed to continue for 20 min then cooled down to room temperature. Due to evaporation, the solution lost 13.31 mL. Considering the loss of water, the final concentration of AgNO<sub>3</sub> was 0.0203 M and for the 3 citrate solutions it was 0.203 M, 0.304 M, and 0.405 M. The powders were collected by centrifugation at 6500 rpm for 40 min. and dried overnight at 65°C.

### Characterization

The morphology of the Ag/MMT powder was observed in a scanning electron microscope (SEM, Hitachi 3-400N and JEOL JSM-6010) and its elemental compositions were shown in the energy dispersive spectroscopy (EDS, JEOL JSM-6010). The structure was investigated by X-ray diffraction (XRD, Shimadzu XRD-7000 Maxima). Specifically, XRD was employed to determine the Ag crystals within the angle range 35° ≤ 2θ ≤ 80°. Ultraviolet-visible spectroscopy (UV-VIS, Shimadzu UVmini-1240) was

used to determine the presence of Ag nanoparticles in solution.

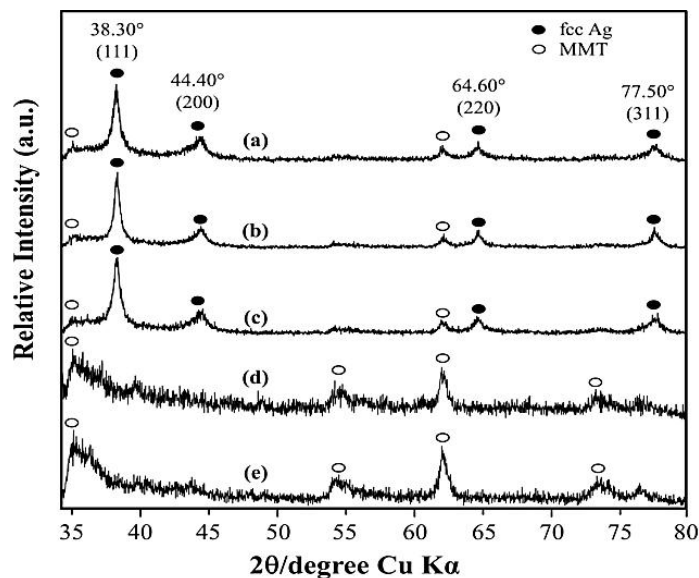
The antibacterial activity of Ag/MMT against *Escherichia coli* and *Staphylococcus aureus* was observed through agar diffusion test performed by the Natural Sciences Research Institute of the University of the Philippines – Diliman. The test organisms were *E. coli* UPCC 1195 and *S. aureus* UPCC 1143. About 3 mm thick Nutrient Agar (NA) plates were inoculated with the microbial suspension by swabbing the entire agar surface for three times. Three equidistant wells with 10 mm diameter were made on the agar plate. 200 µL of the sample was placed in each well. For the positive control, one well at the center of the agar plate was placed with 6 mm Chloramphenicol (30 µg) disc. The NA plates were incubated at 35°C for 24 hrs. After the incubation, the clearing zones were measured in millimeters.

## 3 Results and Discussion

In this study, Ag ions are reduced to Ag NPs via chemical reduction using sodium citrate as the reducing agent and MMT as the stabilizer. The reduction reaction proceeded according to the following equation.[23]



Figure 1 shows the XRD patterns of the sample from 2θ = 35 – 80°. It can be noted that all reduced samples as in Figure 1a-c shows a distinct peak at around 2θ = 38.30°, and minor peaks at 2θ = 44.40°, 64.60° and 77.50° which corresponds to the peaks 111, 200, 220, and 311 of the metallic Ag, respectively.[17] The average size of the Ag crystallite based on the 111 peaks was calculated using the Scherrer equation where k = 0.94. The crystallite size for 0.203 M, 0.304 M and 0.405 M are 13.29 nm, 16.33 nm and 12.54 nm respectively.



**Figure 1.** XRD Patterns of (a) Ag-MMT with 0.20 M citrate, (b) Ag-MMT with 0.30 M citrate, (c) Ag-MMT with 0.40 M citrate, (d) Ag+/MMT, and (e) MMT

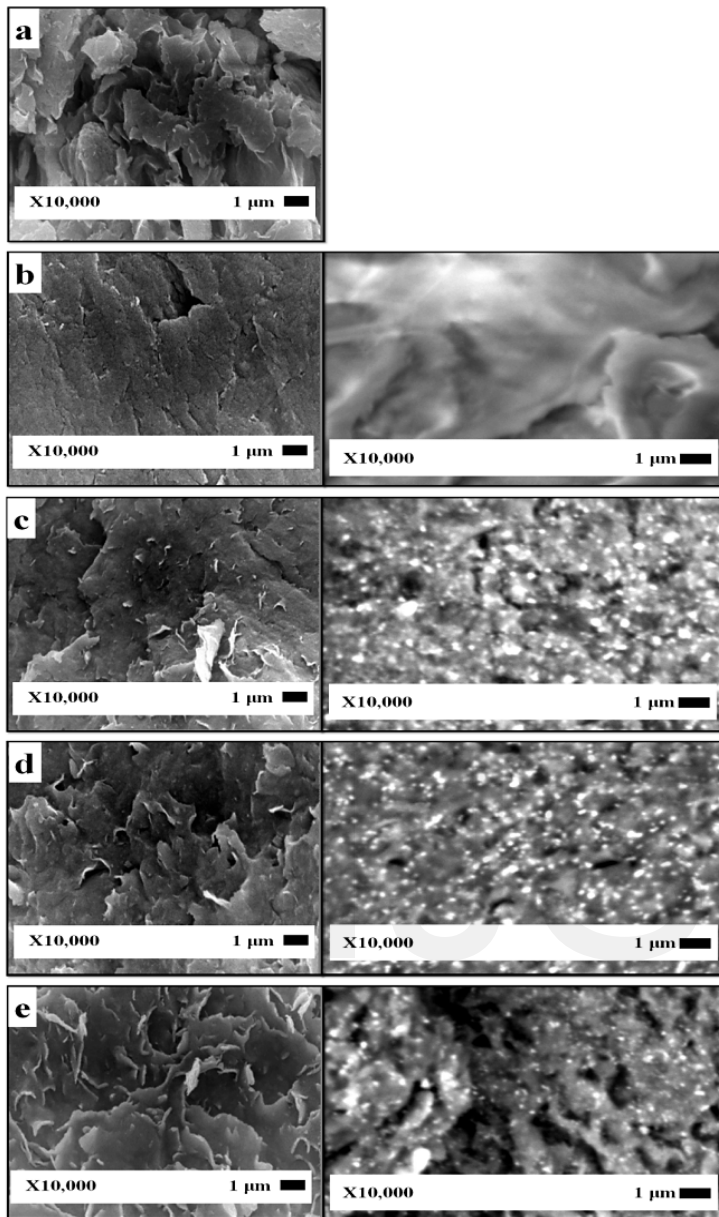
Figure 2 shows the SEM images of MMT indicating changes on its surface morphology upon addition of reducing agent. Figure 2a shows layered structures with flakes which is a characteristic of MMT. Figure 2b displays Ag+/MMT having flatter surface as compared to the original MMT caused by MMT swelling upon introduction of Ag+. Figure 2c-e showed exfoliated structures at all citrate concentration. As citrate concentration increased, flakes on the surface had rougher and sharper edges caused by reduction of Ag on the surface. Backscattered images of Ag loaded MMT from Figure 2 indicated well distributed Ag nanoparticles on the surface of MMT, although there are sites of visible agglomeration. The Ag nanoparticles are identified as the shiny particles on the surface of MMT.

At 0.203 M citrate concentration, the size of Ag nanoparticles ranged from 60 – 284 nm, with a mean particle size of 128.19 nm. At 0.304 M citrate concentration, the particle size ranged from 41 – 248 nm, with a mean of 103.90nm. At 0.405 M citrate concentration, the particle size ranged from 37 – 208 nm nm with a mean of 79.02 nm. The mean diameters for all reduced samples are larger than the computed crystallite size from Scherrer equation which means that the Ag nanoparticles particles are polycrystalline. Agglomeration of nanoparticles also contributed to the large apparent particle size.

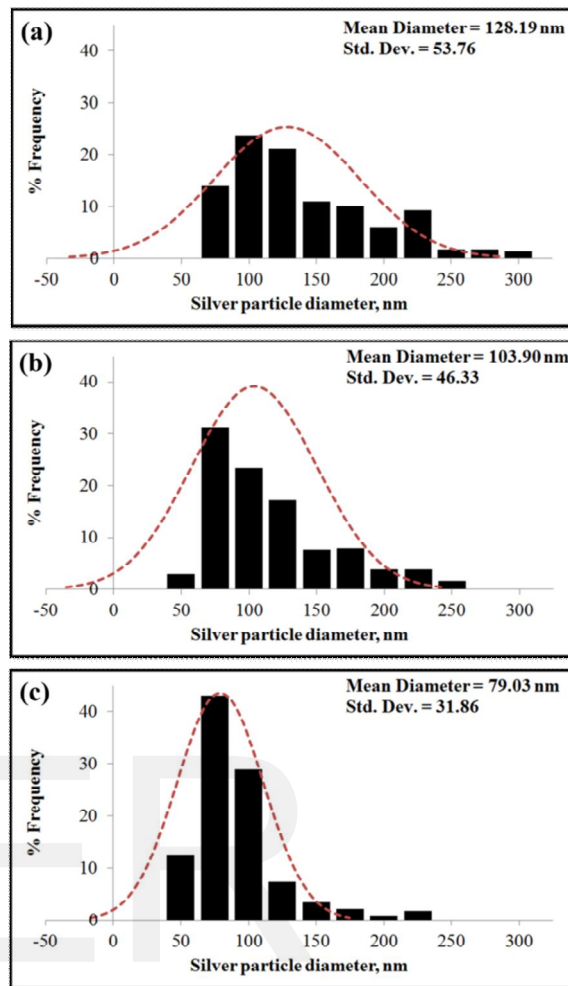
When the concentration of citrate is increased, the number of nucleation sites are also increased. As a result,

smaller Ag nanoparticles with narrower size distribution are produced. From the histogram in Figure 3, it was shown that particle size distribution was more uniform as citrate concentration was increased based on the decreased standard deviation. It can be noted that 0.405 M citrate produced the most uniform particle size distribution with a standard deviation of 31.79 and as exhibited by its sharper distribution curve.

EDS results for MMT, Ag+/MMT and Ag-MMT with 0.302 M citrate are shown in Figure 4. Figure 4a confirms the presence of Na in the original MMT sample. Figure 4b shows several peaks for element Ag, verifying its introduction to MMT. Figure 4c shows the same peaks for Ag which means that upon addition of citrate, the reduced Ag remains on MMT and was not displaced onto the solution.



**Figure 2.** SEM micrographs of (a) MMT, (b) Ag+/MMT, (c) Ag-MMT with 0.203 M citrate, (d) Ag-MMT with 0.304 M citrate and (e) Ag-MMT with 0.405 M citrate



**Figure 3.** Particle size distribution of Ag loaded MMT formed at different citrate concentration of (a) 0.203 M, (b) 0.304 M, (c) 0.405 M

The inhibition zone for the Ag-MMT sample with 0.405 M citrate was tested against gram negative *E. coli* and gram positive *S. aureus*. The inhibition zone is presented in Figure 5. The results showed that the sample was not able to inhibit the growth of *E. coli*. However, it showed antimicrobial activity against *S. aureus* with an antimicrobial index of 0.4. The failure of the sample to inhibit *E. coli* can be attributed to the presence of Lipopolysaccharides (LPS) molecules which is a characteristic trait of gram negative bacteria. LPS surrounds the gram negative bacteria and acts as the second outer membrane which provides an extra layer of protection by serving as a selective permeability barrier.[24, 25] Gram positive *S. aureus* does not have the LPS molecules hence it is easier for the nanoparticles to anchor to the bacteria, eventually leading to their growth inhibition.



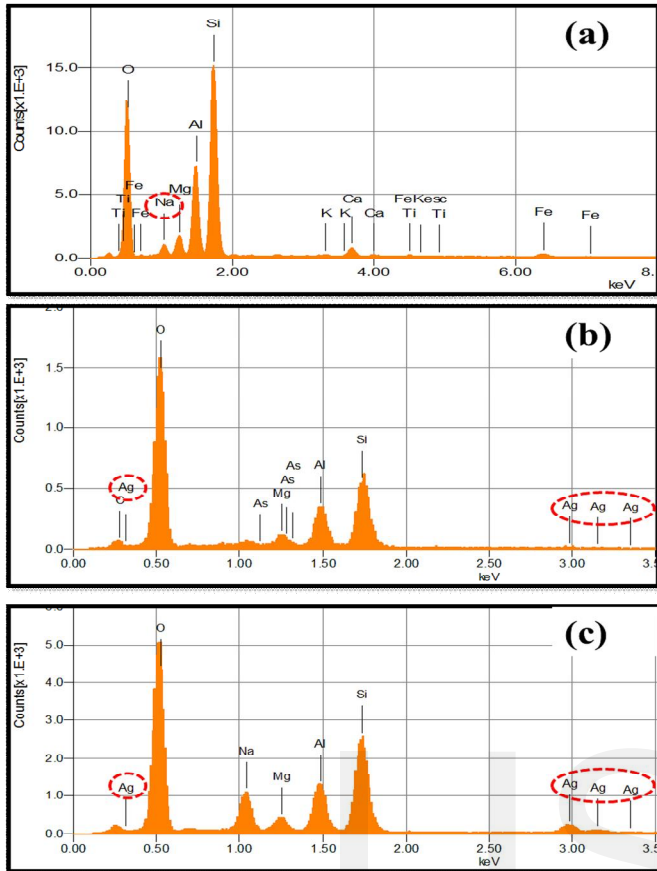


Figure 4. EDS Spectra of (a) MMT, (b) Ag+/MMT, and (c) Ag-MMT with 0.203 M citrate

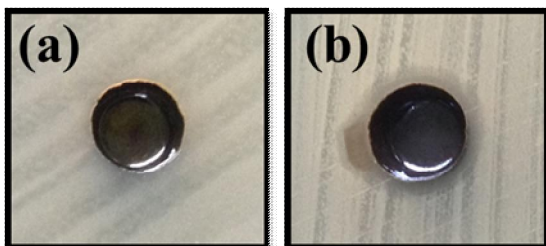


Figure 5. Inhibition Zone Test for (a) *E. coli* and (b) *S. aureus*

#### 4 Summary

In summary, Ag nanoparticles on montmorillonite clay were synthesized by chemical reduction using citrate. The presence of the Ag nanoparticle was detected at 402-408nm from the peak of the plasmon absorption of the colloidal solution by UV-Vis Spectroscopy. The absorbance peaks are independent of the citrate concentration used to reduce Ag(I). XRD and EDS data shows peaks for Ag, confirming its presence in the montmorillonite.

The surface morphology of montmorillonite show the presence of Ag nanoparticles on the surface, with apparent mean particle size increasing from 79-128 nm as the citrate concentration is decreased. These values are quite larger than the crystallite size estimated from the broadening of the 111 Ag peaks. The calculated values are from 13-16 nm. The agar diffusion test proved the efficacy of Ag-MMT against *S. aureus* with an antimicrobial index of 0.4. Its failure to inhibit *E. coli* may be attributed to the presence of LPS molecule of gram negative bacteria, which acts as a selective permeability barrier.

Further studies can be made on the intercalation of Ag NPs in MMT interlayers. Change in the basal spacing of MMT could be the basis whether intercalation is observed. Further study could show intercalation of Ag NPs on interlayers with different reducing agents. Furthermore, the antimicrobial activity of synthesized nanoparticles intercalated in MMT could be compared to that of nanoparticles adsorbed on the surface only.

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