Science Diliman (July-December 2015) 27:2, 10-20

Formation of Highly Antimicrobial Copper Nanoparticles by Electroless Deposition in Water

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ABSTRACT

Metallic copper (Cu)nanoparticles (CuNPs)with mean diametersranging from 37 nm to 44 nm were synthesized by electroless deposition (chemical reduction)in an aqueous solution at 353 K. Cupric oxide (CuO) powder, which has low solubility in water, was used as the Cu(II) precursor. Gelatin and hydrazine (N_2H_4) were employed as the protective agent and reductant, respectively. Small spherical Cu nanoparticles having mean diameter of 37 nm were formed using 2.25 wt% gelatin. In the absence of gelatin, large Cu nanoparticles of 377 nm in mean diameter were produced. Both cuprous oxide (Cu_2O) and metallic Cu peaks were identified from the X-ray diffraction pattern of the samples. The results suggest that gelatin hinders the growth of Cu nanoparticles in solution and protects the nanoparticles from oxidation. Interestingly, the as-prepared Cu nanoparticles exhibit strong antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.

Keywords: Copper nanoparticles, electroless deposition, hydrazine antimicrobial

LAYMAN'S ABSTRACT

Spherical copper (Cu) nanoparticles with average diameter in the range of 37-44 nm were formed by simple chemical reduction in water at 80°C. Gelatin was used to protect the Cu nanoparticles from oxidation and prevent their agglomeration in solution. In fact, larger Cu nanoparticles of about 377 nm in average diameter were produced when gelatin was absent in the solution. In addition, oxides of Cu (Cu_2O) were observed in the X-ray diffraction pattern of the same sample.

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ISSN 0115-7809 Print / ISSN 2012-0818 Online

INTRODUCTION

Metal nanoparticles are widely gaining recognition due to their unique properties, which result to diverse applications in the fields of microelectronics and biotechnology(Usman et al. 2012). In particular, silver (Ag)and copper (Cu) nanoparticles are drawing attention due to their excellent thermal, electrical, catalytic, and optical properties. For example, Ag and Cu nanoparticles have been shown to exhibit superior bactericidal effect because of their large surface areas, which allow them to closely interact with bacteria. Thus, these nanomaterials could be potential antimicrobial agents for antibiotic-resistant microorganisms, which are alarmingly becoming more widespread (Ruparelia et al. 2008; Zhang and Yang 2013; Chatterjee et al. 2014).

The antimicrobial properties of Ag nanoparticles have been extensively studied over the years. Many hypotheses that attempt to elucidate the mechanism of its antimicrobial activity have been proposed. Ag nanoparticles can penetrate the cell membrane and can result to the leakage of intracellular substances, ultimately leading to cell death (Ruparelia et al. 2008). On the other hand, recent studies have shown that Cu nanoparticles could also be a promising antimicrobial agent, and hence, a cheaper alternative to Ag (Bogdanovic et al. 2014; Chatterjee et al. 2014; Shankar and Rhim 2014). Cu has the ability to act as an electron donor or acceptor depending on its oxidation state. Cu can easily change between oxidation states because of its high electrochemical potential, allowing it to interact freely with bacterial proteins (Konieczny and Rdzawski 2012). For instance, the oxidation of Cu^{+} generates hydroxyl radicals in Fenton's reaction as in Equation (1). The generated hydroxyl radicals consequently breakdown bacterial proteins and DNA (Hajipour et al. 2012).

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{-} + OH^{-} \qquad (1)$$

Rispoli et al. (2010) investigated the antimicrobial activity of Cu nanoparticles against *Escherichia coli* (*E. coli*) and found out that the toxicity of Cu nanoparticles against *E. coli* is dependent on temperature, aeration rate, pH, and the concentration of Cu nanoparticles. Moreover, a separate study has shown that the antimicrobial activity of Cu nanoparticles is more effective against bacteria than fungi (Ramyadevi et al. 2012). Cu nanoparticles have also been incorporated in natural fibers and tested for their bactericidal effect against *E. coli* and *Staphylococcus aureus* (*S. aureus*) (Chowdhury et al. 2013). The Cu nanoparticles immobilized in natural fibers exhibited 7% antifungal activity and greater bactericidal effect against *E. coli* compared with *S. aureus*.

Cu nanoparticles can be synthesized by numerous methods, such as the polyol process (Zhang et al. 2014), sonochemical method (Dhas et al. 1998), nanosphere lithography (Chan et al. 2007), thermal reduction(Habibi and Kamrani 2010), laser ablation (Sadrolhosseini et al. 2013), hydrothermal synthesis (Giannousi et al. 2014), and electroless deposition (chemical reduction) (Yagi et al. 2008; Yagi et al. 2009; Tan et al. 2014). Though the fabrication of Cu nanoparticles can also be carried out in gas or solid phases, the liquid phase via the "liquid-phase reduction" is the most preferred due to its simplicity (Yagi et al. 2008; Yagi et al. 2009; Magdassi et al. 2010; Tan et al. 2014). Synthesis of Cu nanoparticles in liquid does not require a vacuum environment and can be done at low temperatures, making the process more cost-effective(Umer et al. 2012). Additionally, the morphology of Cu nanoparticles can be easily controlled in solution by varying parameters, such as temperature, pH, and concentrations of the reacting species (Usman et al. 2012). Though many studies have reported the preparation of Cu nanoparticles in solution, it remains a challenge, particularly in water, due to the high propensity of Cu nanoparticles for oxidation (Yagi et al. 2008; Yagi et al. 2009; Tan et al. 2014).

In this study, oxidation-stable Cu nanoparticles were prepared by electroless deposition (chemical reduction) in an aqueous solution using gelatin as protective agent. The effect of gelatin concentration on the particle size of Cu nanoparticles was investigated. The use of gelatin as protective agent has been previously reported (Chatterjee et al. 2012; Zhang and Yang 2013). By contrast to the use of Cu(II) salts in the said studies, the present work used cupric oxide (CuO) as the Cu(II) precursor. CuO has low solubility in aqueous solution, particularly at high pH, favoring the formation of small and uniform Cu nanoparticles.The oxidation of Cu nanoparticles in solution was monitored by UV-Vis spectroscopy, while the antimicrobial activity of Cu nanoparticles against *E. coli* and *S. aureus* was determined by the agar disk diffusion method.

MATERIALS AND METHODS

Materials

Reagent grade cupric oxide powder (CuO, HiMedia Labs), 98 wt% hydrazine solution in water (N_2H_4, Sigma Aldrich Inc.), sodium hydroxide pellets (NaOH, RSI Labscan), and gelatin granules (Nacalai Tesque Inc.) were utilized as received. Deionized water was used throughout the synthesis.

Electroless Deposition of Cu Nanoparticles

CuO suspension solution was prepared by dispersing 4.87 g of CuO powder in 42 mL deionized water by sonication. N-$_2H_4$solution was prepared by dissolving 3.06 g of N_2H_4 in 42 mL of deionized water. Afterwards, 18 g of 0% to 15% gelatin solution in water was added to both the CuO suspension and theN_2H_4 solution. The pH values of the two solutions were adjusted to 12 at room temperature using 1.5 M NaOH aqueous solution. Nitrogen (N_2) gas was introduced into the solution 30 mins prior to the reaction to eliminate dissolved oxygen. The N_2H_4 solution was then slowly added to the CuO suspension while stirring at 500 rpm. The total solution was allowed to react for 2 h at 353 K under continuous nitrogen gas purging. The as-prepared CuNPs were collected by centrifugation and washed by deionized water several times.

Characterization

The size and morphology of the Cu nano particles were observed using a scanning electron microscope with an acceleration voltage of 10 kV (SEM, Hitachi SEM S-3700N). Image analyses of 300 nanoparticles from several SEM images were used to determine the mean particle size. The structure of the Cu nanoparticles was investigated in an X-ray diffractometer using a voltage of 40 kV and a current of 30 mA (XRD, MAXima_X XRD-700). The ultraviolet-visible (UV-Vis) absorption spectra of the Cu nanoparticles were obtained using a UV-Vis spectrometer (Shimadzu UV-1700) within the wavelength range of 400 nm to 800 nm.

Antimicrobial Activity

Gram-positive bacteria *S. aureus* (MTCC-3160) and Gram-negative bacteria *E. coli* (MTCC-2642) were suspended in 0.1% peptone water and inoculated on 3-mm thick nutrient agar plates. A total of 200 µL of the Cu nanoparticle solutions were added on three equidistant wells with diameters of 10 mm on the nutrient agar plates. Antibiotic discs were used on the center of the nutrient agar plates as the positive control. Nutrient agar plates were then incubated at 308 K for 24 h. The average diameters of the clearing zones were measured, and the antimicrobial index (AI) was calculated using the formula below:

$$\mathrm{AI} = \frac{\text{Diameter of clearing zone} - \text{Diameter of well}}{\text{Diameter of well}} \qquad (2)$$

RESULTS AND DISCUSSION

Electroless Deposition (Chemical Reduction) of Copper Nanoparticles

Figure 1 shows the SEM images of Cu nanoparticles formed by electroless deposition (chemical reduction) in an aqueous solution using increasing concentrations of gelatin. In the absence of gelatin, large Cu particles with mean diameter of 377 nm were formed in the solution. The particles were irregularly shaped and agglomerated as seen in Figure 1a, possibly due to the lack of steric hindrance by gelatin. On the other hand, addition of 0.75 wt% gelatin to the reaction suspension yielded spherical Cu nanoparticles with mean diameter of 44 nm.

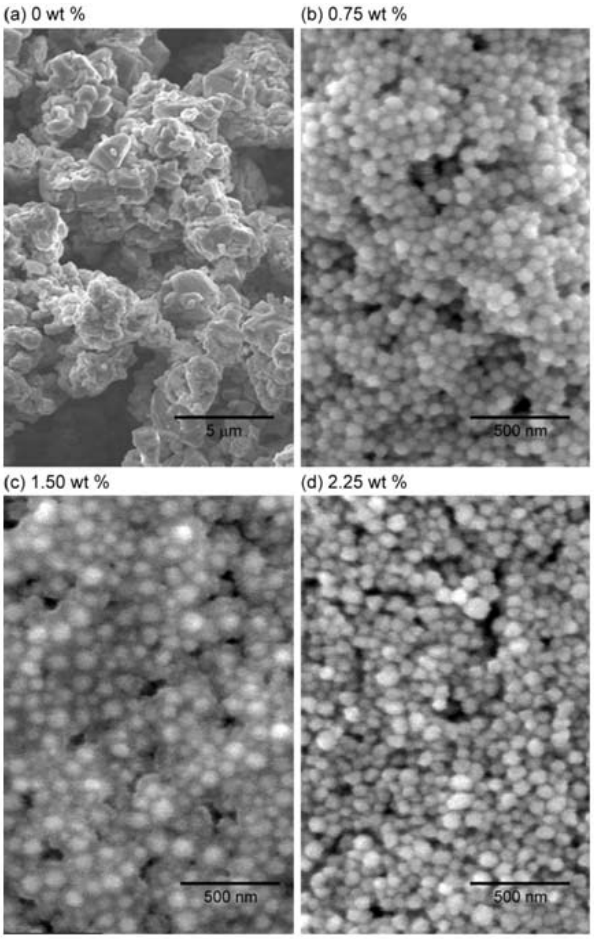

Figure 1. SEM images of Cu nanoparticles prepared by electroless deposition (chemical reduction) at 353 K in aqueous solution using (a) 0 wt%, (b) 0.75 wt%, (c) 1.50 wt%, and (d) 2.25 wt% gelatin as protective agent.

When the amount of gelatin was increased to 1.50 wt% and 2.25 wt%, smaller Cu spherical nanoparticles with mean diameters of 37 nm to 38 nm were generated. However, the smaller Cu nanoparticles were surrounded by an organic layer, probably gelatin, as seen in Figure 1c. The presence of the organic layer suggests that the washing process was insufficient to remove the excess gelatin around the Cu nanoparticles.

Except for the sample prepared without gelatin, the spherical Cu nanoparticles were uniform in size with standard deviations less than 10% of the mean particle size. It is possible that gelatin effectively restricts the growth of Cu nanoparticles in solution. As a result, uniform and minute Cu nanoparticles were formed. Adherence of gelatin on the surface of Cu nanoparticles also increases the space steric hindrance among the nanoparticles, effectively inhibiting agglomeration (Magdassi et al. 2010; Zhang and Yang 2013; Tan et al. 2014).

Figure 2 shows the corresponding XRD patterns of Cu nanoparticles formed with increasing amount of gelatin. For the samples with gelatin, peaks at 43.44°, 50.62°, and 74.20° were attributed to 111, 200, and 220 peaks of face-centered (fcc) Cu, respectively. The presence of these peaks indicates that only metallic Cu was formed in the solution. The Cu peaks also appear broadened compared to the sample

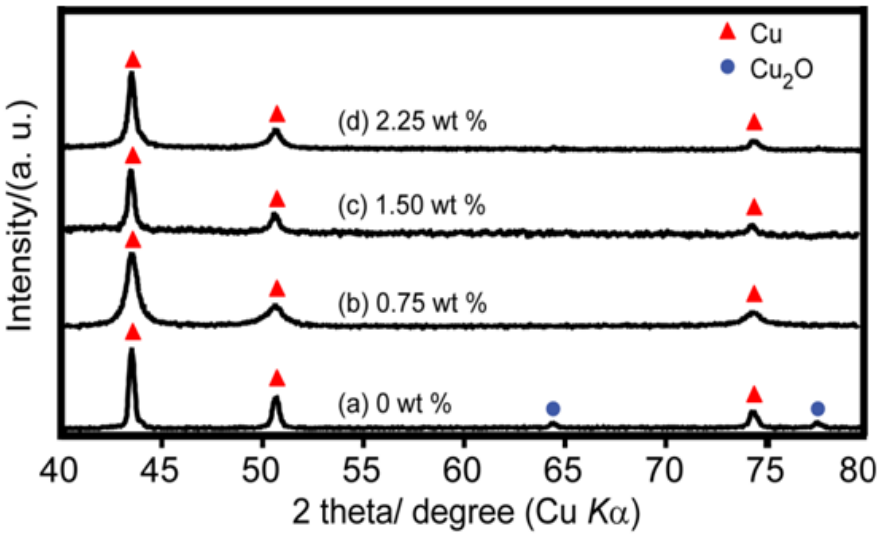

Figure 2. XRD patterns of Cu nanoparticles synthesized by electroless deposition (chemical reduction) at 353 K using (a) 0 wt%, (b) 0.75 wt%, (c) 1.50 wt%, and (d) 2.25 wt% gelatin as protective agent.

without gelatin, suggesting smaller crystallite size. Such observationis in agreement with the apparent diameter determined from the SEM images in Figure 1. In the absence of gelatin, peaks of Cu_2O at 2q = 64.22° and 77.42°, which are reflections of the 220 and 222 planes, respectively, were indexed together with peaks of fcc Cu as shown in Figure 2a. Oxidation of Cu nanoparticles possibly occurred in the absence of gelatin, explaining the Cu_2O peaks (Tan et al. 2014).

Figure 3 shows the UV-Vis spectra of Cu nanoparticles generated using 2.25 wt% gelatin after 0 to 60 days of storage in aqueous solution. This particular sample was selected for the oxidation study due to its small particle size of 37 nm in mean diameter. The small particle diameter suggests large surface-to-volume ratio and a high tendency for oxidation. The as-prepared Cu nanoparticles exhibit an absorption peak at about 584 nm, a value well within the range of the Cu absorption peak (573 nm to 600 nm). Interestingly, there were no significant changes in the Cu absorption peak after 14 to 60 days of storage. No other absorption peak besides that of Cu was observed after 60 days, suggesting that neither agglomeration nor oxidation occurred for the Cu nanoparticles in the solution. Such result is significant for its future applicationas an antimicrobial agent.

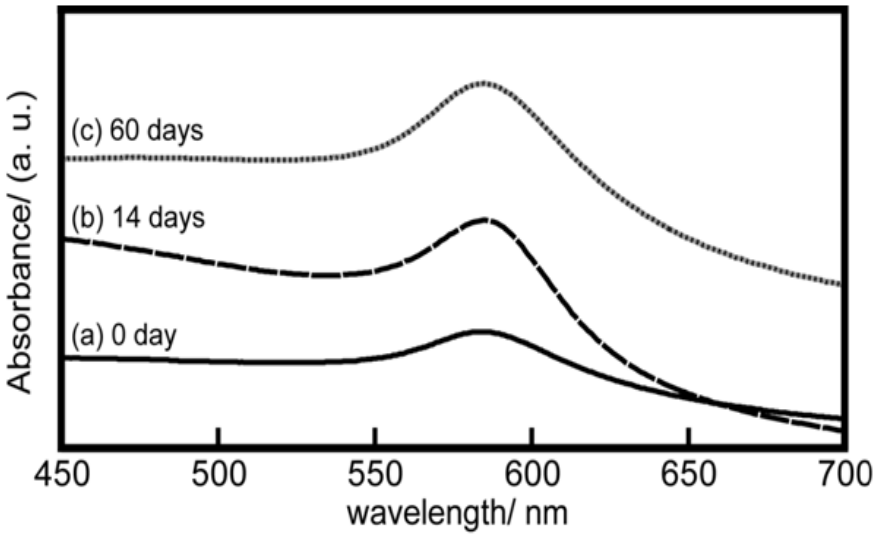

Figure 3. UV-Vis absorption spectra of Cu nanoparticles prepared by electroless deposition (chemical reduction) with 2.25 wt% gelatin after (a) 0,(b) 14, and (c) 60day(s) of storage.

Antimicrobial Activity of Cu Nanoparticles

Figure 4 shows the actual images of the nutrient agar plates containing *E. coli* and *S. aureus* inoculates incubated with200 µL of theas-prepared Cu nanoparticles with mean diameters of 38 nm to 377 nm. Compared to the samples with larger particle diameters, Cu nanoparticles with mean diameter of 38 nm exhibit higher average inhibition zone at 55 mm and an antimicrobial index of at least 4.5. Such performance

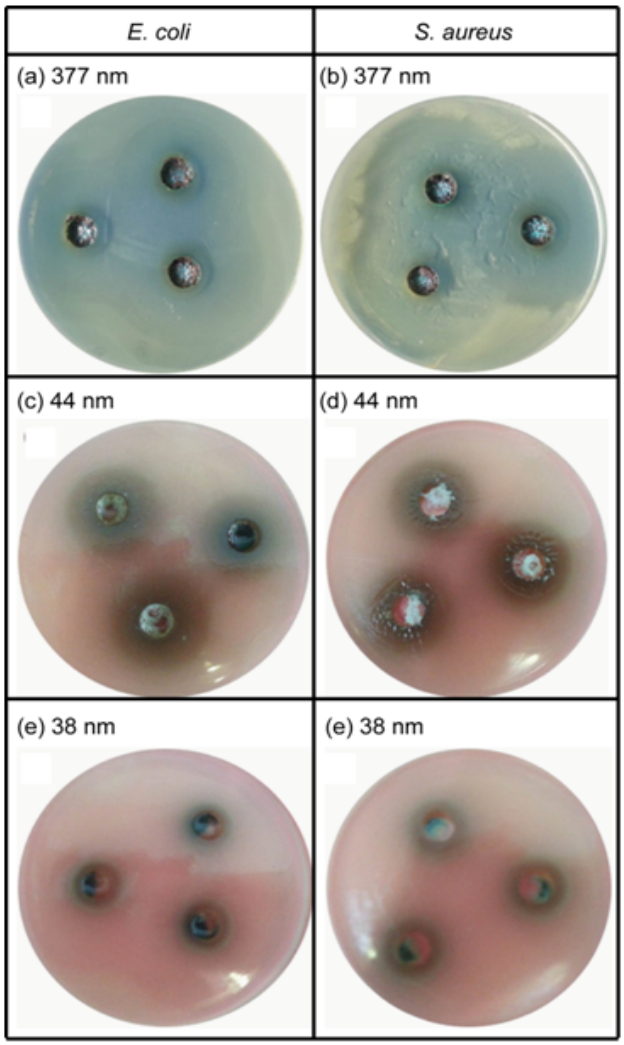

Figure 4. Clearing zones of 200 µL Cu nanoparticles with mean particle sizes of (a-b) 377 nm, (c-d) 44 nm, and (e-f) 38 nm against Gram (-) *E. coli* and Gram (+) *S. aureus*.

can be attributed to the large specific surface area of these Cu nanoparticles. Hydroxyl radicalsare generated from the interaction of Cu with the bacteria, leading to its high antimicrobial activity by oxidative damage to the bacteria (Ruparelia et al. 2008; Chatterjee et al. 2014). Though the 377-nm Cu nanoparticles have lower antimicrobial properties than the smaller ones, they exhibit antimicrobial properties comparable to chloramphenicol, which was used as the positive control. The antimicrobial properties of the as-prepared Cu nanoparticles against *E. coli* and *S. aureus* are summarized in Table 1.

Table 1. Comparison of the antimicrobial activities of the synthesized Cu nanoparticles against Gram (-) *E. coli* and Gram (+) *S. aureus*

Test Organism	Cu Nanoparticles Diameter (nm)	Average Inhibition Zone (mm)	Antimicrobial Index (AI)
	38	55	4.5
Gram (-)	44	53	4.3
Escherichia coli	377	38.3	3.2
	Chloramphenicol*	25	4.0
	38	>55	>4.5
Gram (+)	44	53.3	4.3
Staphylococcus aureus	377	42.3	3.2
	Chloramphenicol*	25	3.2

*6-mm disc contains 30 µg Chloramphenicol

CONCLUSION

Spherical Cu nanoparticles with mean diameters of 37 nm to 44 nm were synthesized via electroless deposition (chemical reduction) using CuO suspension in water. The presence of gelatin as protective agent provides excellent oxidation and agglomeration protection for the Cu nanoparticles. The particle size of Cu nanoparticles was controlled to some extent by changing the concentration of the gelatin in the solution. Smaller Cu nanoparticles were generally formed with higher gelatin concentration. This observation can be attributed to the steric hindrance provided by the gelatin, which thereby inhibits particle growth. Oxidation studies exhibited that minute Cu nanoparticles of 37 nm in mean diameter were stable against oxidation even after 60 days of storage. The Cu nanoparticles also exhibited high microbial activity against Gram (-) *E. coli* and Gram (+) *S. aureus* with clearing zones rangingfrom 38 nm to 55 mm in diameter. Further study is needed, in order to fully elucidate the antimicrobial property of the as-prepared Cu nanoparticles.

ACKNOWLEDGMENTS

This study was supported by the University of the Philippines under the Office of the Vice-Chancellor for Research and Development (PhD Incentive Award), the Department of Science and Technology - Philippine Council for Industry, Energy and Emerging Technology Research and Development (DOST-PCIEERD), and the College of Engineering Professorial Chair Award (Uratex).

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