Biological synthesis of copper nanoparticles using plant extract

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ABSTRACT

Copper nanoparticles were biologically synthesized using plant leaf extract as reducing agent. On treatment of aqueous solution of $CuSO_4 \cdot 5H_2O$ with Magnolia leaf extract, stable copper nanoparticles were formed. UV-vis spectroscopy was used to monitor the quantitative formation of copper nanoparticles. The synthesized nanoparticles were characterized with inductively coupled plasma spectrometry (ICP), energy dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), and high-resolution transmission electron microscopy (HR-TEM). Electron microscopy analysis of copper nanoparticles indicated that they ranged in size from 40 to 100 nm. Antibacterial tests were carried out by counting viable *Escherichia coli* cells after 24 h growth in shake flasks containing latex foams coated with copper nanoparticles. As a result, foams coated with biologically synthesized copper nanoparticles showed higher antibacterial activity compared with foams untreated. As possible ecofriendly alternatives to chemical and physical methods, biologically synthesized nanoparticles using plant extracts may have applications in various human body-contacting areas.

Keywords: biological synthesis, copper nanoparticles, plant extract, antibacterial activity

1 INTRODUCTION

Nanoparticles present a higher surface to volume ratio with decreasing size of nanoparticles. Specific surface area is relevant for catalytic reactivity and other related properties such as antimicrobial activity in silver nanoparticles [1]. Value of nanomaterials increases from the bulk materials due to change of unique physical, mechanical, optical and electromagnetic properties. For example, the sales costs of silver and gold increase from $95/lb silver and $6,650/lb gold for standard grades, to $415/lb silver and $26,000/lb gold for nanoscale grades [2].

Production of nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production of nanoparticles. However, some chemical methods can not avoid the use of toxic chemicals in the synthesis protocol. Since metal nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticle synthesis that do not use toxic chemicals. Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods [3,4].

Using plants for nanoparticle synthesis can be advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale nanoparticle synthesis [5]. Gardea-Torresdey et al. [6,7] demonstrated gold and silver nanoparticle synthesis within live alfalfa plants from solid media. Extracellular nanoparticle synthesis using plant leaf extracts rather than whole plants would be more economical owing to easier downstream processing. Pioneering works on nanoparticle synthesis using plant extracts have been carried out by Sastry and others [5,8-14].

In most reports on nanoparticle synthesis using plant extracts, the times required for conversion of Ag^+ and Au^{3+} ions to Ag and Au nanoparticles were several hours, which was longer than those of chemical synthesis [5]. If biological synthesis of nanoparticles can compete with chemical methods, there is a need to achieve faster synthesis rates. We carried out engineering approaches such as rapid nanoparticle synthesis using plant extracts and size and shape control of the synthesized nanoparticles [14-18].

The antimicrobial properties of silver nanoparticles are well-established [19-21] and several mechanisms for their bactericidal effects have been proposed. Although only a few studies have reported the antibacterial properties of copper nanoparticles, they show copper nanoparticles have a significant promise as bactericidal agent [22]. However, other nanoparticles, such as platinum, gold, iron oxide, silica and its oxides, and nickel have not shown bactericidal effects in studies with *Escherichia coli* [23]. Yoon et al. [24] reported the antibacterial effects of silver and copper nanoparticles using *E. coli* and *Bacillus subtilis*, where the copper nanoparticles demonstrated superior antibacterial activity compared to the silver nanoparticles. Silver and copper nanoparticles supported on various suitable materials, such as carbon, polyurethane foam, polymers and sepiolite have also been effectively used for bactericidal applications [20,21,25-27]. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution [28].

In this study, we report for the first time biological synthesis of stable copper nanoparticles using plant leaf extract. We investigated the effects of reaction conditions such as reaction temperature, leaf broth concentration, and

$CuSO_4 \cdot 5H_2O$ concentration on synthesis rate and particle size of the copper nanoparticles. We also carried out antibacterial test of the biosynthesized copper nanoparticles by coating on latex foam products.

2 MATERIALS AND METHODS

2.1 Materials

Copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$; 99.5% purity) was purchased from Samchun Chemical (Korea). Latex foam was supplied from Latex Korea Co.

2.2 Biological Synthesis and Characterization of Copper Nanoparticles

Magnolia kobus leaves were collected and dried for 2 days at room temperature. The plant leaf broth solution was prepared by taking 25 g of thoroughly washed and finely cut leaves in a 1 L beaker with 500 ml of sterile distilled water and then boiling the mixture for 5 min before finally decanting it. They were stored at 4 ^oC and used within a week.

Typically, 30 ml of leaf broth was added to 170 ml of 1 mM aqueous $CuSO_4 \cdot 5H_2O$ solution for the reduction of Cu ions. The effects of temperature on synthesis rate and particle size of the prepared copper nanoparticles were studied by carrying out the reaction in water bath at 25 - 95 ^oC with reflux. The structure were analyzed by scanning electron microscopy (SEM, Hitachi S-2500C) and high-resolution transmission electron microscopy (HR-TEM; JEOL-2010). Copper concentrations were determined using inductively coupled plasma spectrometry (ICP, JY38Plus).

2.3 Chemical Synthesis of Copper Nanoparticles

4 ml of Tween 20 and 6 ml of sodium borohydride was added to 190 ml of 1 mM aqueous $CuSO_4 \cdot 5H_2O$ under 300 rpm stirring. The reaction medium was allowed to react for 2 hour at 25 ^oC.

2.4 Antibacterial Activity

Latex foams were washed, sterilized and dried before use. Latex foam (2 × 2 × 2 cm) was added in solution containing copper nanoparticles synthesized with 1 mM $CuSO_4 \cdot 5H_2O$ and 15% *Magnolia kobus* leaf broth. The weight of latex foam was 5 g and the total volume of solution was 100 ml. The copper colloid solution containing latex foam was placed in shaking incubator for 1 hr at 37 ^oC. The resulting foams coated with copper nanoparticles were dried in oven for 24 hr at 50 ^oC.

Antibacterial activity was tested against gram-negative *E. coli* strain (ATCC 25922). Cells were grown in LB media containing latex foams for 24 hr at 37 ^oC. Then 0.1 ml of cultured solution was transferred to agar medium and incubated for another 24 h. Colonies of viable microbes were counted. Antibacterial activity was calculated by comparing with the colony count of control (untreated foams).

3 RESULTS AND DISCUSSION

Reduction of the copper ion to copper nanoparticles during exposure to the plant leaf extract could be followed by color change and thus UV-vis spectroscopy. It is observed that the maximum absorbance occurs at ca. 560 nm and steadily increases in intensity as a function of reaction time. We quantitatively monitored the concentrations of copper nanoparticles and conversion by measuring the absorbance at 560 nm. The linear relationship was obtained between the copper concentration determined by ICP and the absorbance at 560 nm.

TEM images obtained with 15 - 20% Magnolia leaf broth and 1 mM $CuSO_4 \cdot 5H_2O$ solution showed that relatively spherical nanoparticles are formed with diameter of 45 - 110 nm. As the reaction temperature increased, both synthesis rate and conversion to copper nanoparticles increased. The conversion after 24 hr was about 70% at 25 ^oC and 80 - 100% at 60 and 95 ^oC. The average particle size decreased from 110 nm at 25 ^oC to 45 nm at 95 ^oC. Regarding the reason of decrease in particle size with temperature, we can hypothesize as follows. As the reaction temperature increases, the reaction rate increases and thus most copper ions are consumed in the formation of nuclei, stopping the secondary reduction process on the surface of the preformed nuclei. Similar trends were observed with gold and silver nanoparticles synthesized using plant extracts [11,14,15].

Effects of different Magnolia leaf broth concentrations at 5 - 20% were investigated on copper nanoparticles formation at 1 mM $CuSO_4 \cdot 5H_2O$. The reaction rate was highest at 20% leaf broth concentration. With increasing the leaf broth concentration, the average particle size decreased up to 15% leaf broth concentration and then increased at 20% leaf broth concentration. Effects of $CuSO_4 \cdot 5H_2O$ concentration were also investigated on conversion to copper nanoparticles obtained with 15% Magnolia leaf broth concentration. The times required for more than 90% conversion were 1600, 1400, and 300 min at 95 ^oC, respectively, when $CuSO_4 \cdot 5H_2O$ concentrations were 0.5, 1, and 2 mM, respectively. The average particle size decreased with increasing the $CuSO_4 \cdot 5H_2O$ concentration. It is considered that particle size is dependent on various conditions such as reaction temperature, leaf broth concentration and $CuSO_4 \cdot 5H_2O$ concentration.

EDS profile showed copper signals along with oxygen and carbon peak, which may originate from the biomolecules that are bound to the surface of the copper nanoparticles. XPS spectrum showed characteristic copper peaks, suggesting that copper nanoparticles were successfully synthesized using Magnolia leaf broth.

Copper nanoparticles were also synthesized using well-known chemical method for comparison. Sodium borohydride and Tween 20 were used as reducing and stabilizing agent, respectively. SEM image showed that spherical nanoparticles were formed with diameter of ca. 150 nm. It was observed that chemically synthesized copper nanoparticles oxidized and settled down after 24 hr, while copper nanoparticles synthesized using plant extract were stable for over 30 days because some capping materials surround the surface of nanoparticles. This is another advantage using plant extract for nanoparticle synthesis over using chemical method.

Latex foams were dip-coated with copper nanoparticles to test antibacterial activity. The color of the treated foams changed to brown with increasing the copper nanoparticle concentration due to the coating of copper nanoparticles on the surface of foams, while the untreated foams were white. Table 1 summarizes antibacterial activities of copper nanoparticles synthesized using Magnolia leaf extract at various temperatures and leaf broth concentrations compared with chemically synthesized copper nanoparticles. The antibacterial activities were 40, 95, and 99%, respectively, when the synthesis temperatures were 25, 60, and 95 ^{o}C, respectively. It was shown that the antibacterial activities were inversely proportional to the average nanoparticle sizes. Chemically synthesized copper nanoparticles at 25 ^{o}C showed 17% of antibacterial activity. When the leaf broth concentrations were 5, 10, 15, and 20%, respectively, the antibacterial activities were 70, 89, 99, and 75%, respectively, which was also inversely proportional to the average nanoparticle sizes.

Synthesis method of copper nanoparticles	**Synthesis temp. (^{o}C)**	**Leaf broth conc. (%)**	**Anti-bacterial activity (%)**	**Average particle size (nm)**
Chemical method	25	-	17	150
Biological method	25	15	40	110
Biological method	60	15	95	90
Biological method	95	5	70	91
Biological method	95	10	89	58
Biological method	95	15	99	37
Biological method	95	20	75	82

Table 1: Summary of antibacterial activity of copper nanoparticles.

4 CONCLUSION

We proposed an ecofriendly method for copper nanoparticle synthesis using plant extract. The conversion to copper nanoparticles was more than 90% using Magnolia leaf broth when the reaction temperature increased to 95 ^{o}C. The particle size ranged from 45 to 110 nm and could be controlled by changing the reaction temperature and leaf broth concentration. The growth of *E. coli* in shake flask culture was significantly suppressed by treatment of copper nanoparticles to the latex foams. The antibacterial activities were inversely proportional to the average nanoparticle sizes. The biologically synthesized copper nanoparticles using plant extract showed higher antibacterial activity than chemically synthesized copper nanoparticles using sodium borohydride and Tween 20. Copper nanoparticles synthesized using plant extract were stable for over 30 days due to some capping materials surrounding the surface of nanoparticles, which is another advantage using plant extract for nanoparticle synthesis over using chemical method. This environmentally friendly synthesized copper nanoparticles can be used as an inexpensive alternative to antibacterial silver nanoparticles.

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