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Antibacterial activity of copper-based particles synthesized using an electroless deposition technique

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Abstract. Copper-based particles were synthesized using an electroless deposition technique. The said synthesis was done in an aqueous solution by reducing copper oxide powders using hydrazine. In this technique, gelatin was used as protective agent. X-ray Diffraction (XRD) measurement shows that the synthesized sample is composed of cuprous oxide (Cu_2O) and copper (Cu) particles. Scanning Electron Microscopy (SEM) shows the morphology of the synthesized copper-based particles. Antimicrobial test shows that the number of *Escherichia coli* organisms reduced to 62.06% after 2 minutes of contact with the sample. Likewise, SEM micrographs of the *Escherichia coli* organisms show that the said organism underwent morphological changes in the presence of the synthesized copper-based particles.

1. Introduction

Copper [1-6] has antibacterial activity against gram-positive and gram-negative bacteria. Copper particles can be incorporated with different systems such as in wastewater treatment to perform its antimicrobial functions. The antimicrobial activity of copper is a function of contact area with microorganisms. Close interaction of copper particles with cells can inhibit the cell's normal function, which could eventually lead to cell death.

The synthesis of metallic copper [7-11] was a challenging task because it can rapidly oxidize in air or in aqueous media. To inhibit or minimize oxidation, copper metals are protected with capping agents or stabilizers. In a case wherein the capping agents do not completely inhibit the formation of oxides, it is important to note that Cu oxide [12-13] also has antimicrobial properties.

The aim of this research is to synthesize copper-based particles using an electroless deposition technique. In this technique, copper oxide powders were reduced using hydrazine in an aqueous solution. This technique used gelatin as protective agent or stabilizer. The antimicrobial activities of the synthesized copper-based particles against *Escherichia coli* were tested.

2. Experimental

2.1. Materials

Copper (II) oxide powders (Nacalai tesque) were used as copper source and lab grade gelatin (Nacalai Tesque) was used as protective agent. Ninety eight weight percent (98 wt%) hydrazine monohydrate ($N_2H_4 \cdot H_2O$, Sigma Aldrich Inc.) and sodium hydroxide pellets (NaOH, Macron Fine Chem) were also used in the synthesis of the copper-based particles.

2.2. Synthesis of Cu-based particles by Electroless deposition

The synthesis of Cu-based particles was prepared according to Balela and Amores [14]. The protective and reducing agent were prepared separately and both were added with 10 wt% gelatin. The pH of reaction mixture was adjusted to 12 by dropwise addition of 1.5 M of NaOH. The total solution was allowed to react for 2 h at 353 K under continuous nitrogen gas purging to prevent oxidation. The Cu-based particles were collected and washed with deionized water.

2.3. Characterization

The morphology of the synthesized Cu-based particles was determined using Scanning Electron Microscopy (JEOL JSM 5310) while the identification of phases present was done using X-ray Diffraction (Shimadzu XRD-7000).

2.4. Antibacterial activity

Contact kill test was used to determine the amount of *Escherichia coli* organisms that remains after the copper-based particles were brought into contact with the test organism. Meanwhile, the effects of copper-based materials on the cell morphology of *Escherichia coli* organisms were observed using Scanning Electron Microscopy (SEM).

3. Results and Discussion

Figure 1 shows the X-ray diffraction pattern of the sample stored for 2 weeks in aqueous solution. Both peaks of cuprous oxide (Cu_2O) and copper (Cu) particles were present in the XRD pattern, suggesting that some of the copper particles oxidized to Cu_2O.

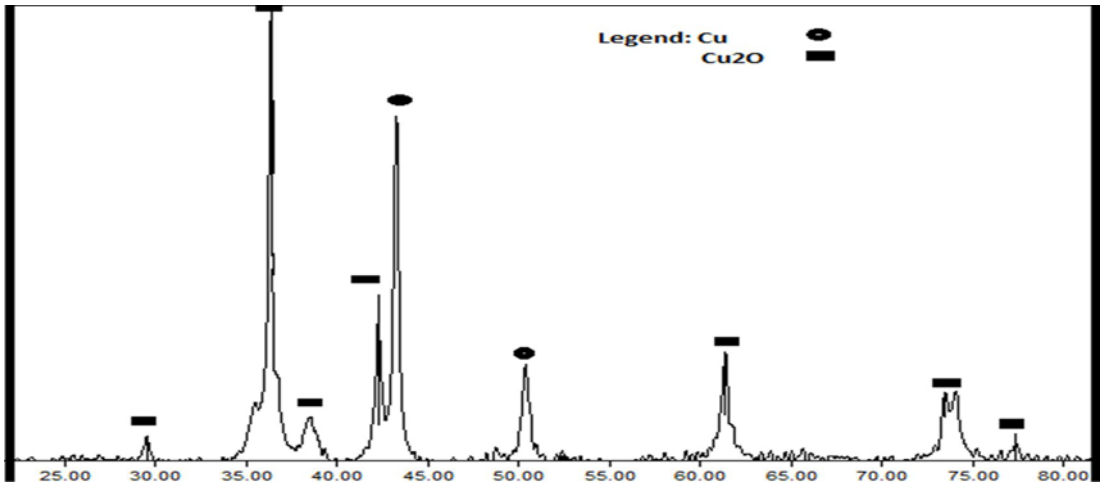

Figure 1. XRD pattern of Cu-based particles after two weeks of storage in aqueous solution

Figure 2 shows the SEM image of synthesized copper-based particles, which was stored in aqueous solution for two weeks. The figure shows that these particles have relatively uniform distribution. The particles appear aggregated but this is just a typical behaviour when they are taken out of solution. This could be also an indication of oxidation of copper to Cu_2O.

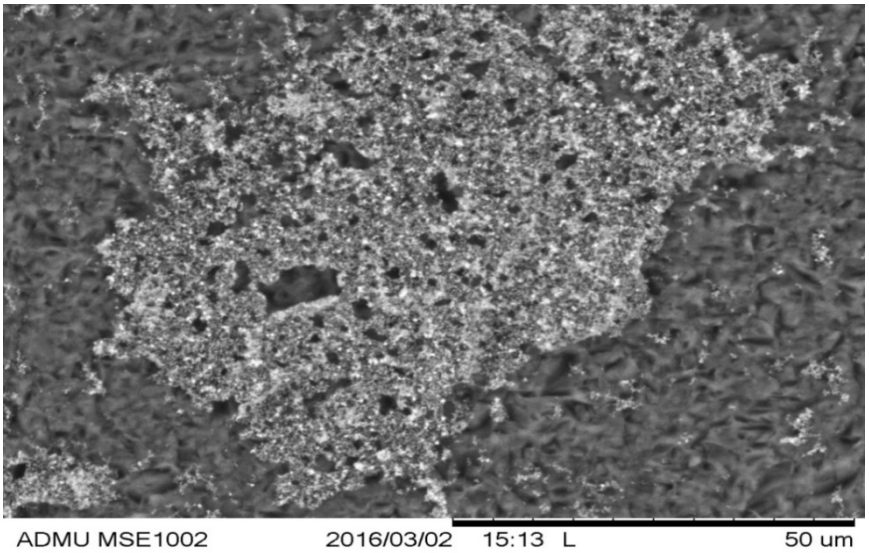

Figure 2. SEM image of Cu-based particles after two weeks of storage in aqueous solution

Table 1 show the result of Contact kill test of Cu-based particles against *Escherichia coli*. The initial count of bacterial samples was 2.3 x 10^6 (CFU/ml) and were reduced to 8.6 x 10^5CFU/ml (62.06%) after two minutes of contact time with the synthesized copper-based particles. The interaction of Cu to outer and inner bacterial membrane might be the possible effect of inhibition of the bacterial samples.

Table 1. Summarize result of Contact kill test against *Escherichia coli.*

Type of Organism	Initial Count	Final Count
Escherichia coli	2.3×10^6 CFU/ml	8.6×10^5 CFU/ml

Figure 3a shows the SEM images of *Escherichia coli* incubated in the absence of copper-based particles. Meanwhile, Figure 3b and Figure 3c show the SEM images of *Escherichia coli* treated with copper-based particles in the presence and absence of light, respectively. Without the copper-based particles, the average size of the test organism is 329 nm. This average size reduces to 152 nm when copper-based particles were introduced in the presence of light. On the other hand, when copper-based particles were introduced in the absence of light, the average size of the test organism reduces to 90 nm.

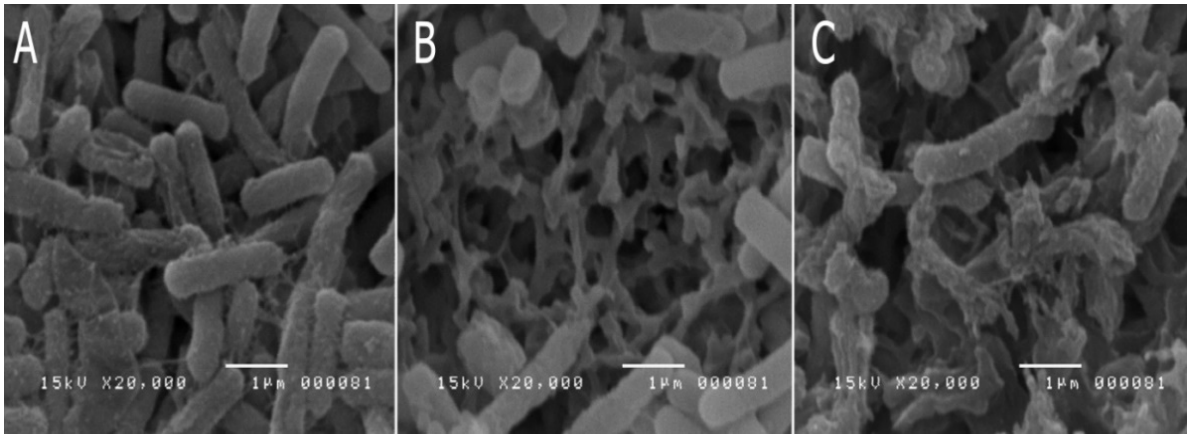

Figure 3. SEM images of (a) *Escherichia coli* organisms and SEM image of *Escherichia coli* organisms treated with the synthesized Cu-based particles in the (b) presence of light and (c) absence of light.

4. Summary and Conclusion

Copper-based particles were synthesized using an electroless deposition technique. The synthesis was done in aqueous solution using copper oxide as copper source, hydrazine as reducing agent and gelatin as protective agent. X-ray diffraction shows that the sample is composed of copper particles and Cu_2O. Scanning electron microscopy shows that the copper-based particles were aggregated. The synthesized copper-based particles show antibacterial activity against *Escherichia coli.* In such, after 2 minutes of contact with the sample, the number of *Escherichia coli* organisms reduced to 62.06%. Furthermore, SEM micrographs of *Escherichia coli* organisms also show that morphological changes happened on the said organisms in the presences of the synthesized copper-based particles.

Acknowledgment

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