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Synthesis of Copper Nanoparticles and its Antibacterial Activity against *Escherichia coli*

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ABSTRACT

The antibacterial activities of copper nanoparticles (Cu-NPs) were studied with respect to gram-negative *E. coli* by measuring the growth curves of bacteria. Bacterial cells were treated with Cu nanoparticles powder and the growth rates were investigated under varying concentrations of Cu nanoparticles and incubation times. *E. coli* was shown to be substantially inhibited by copper nanoparticles. These results suggest that Cu-NPs could be used as an effective antibacterial material. The analysis of Kruskal-Wallis test of microbiological data showed that the microbiological bacterial death differ between the different NPs concentration ($p < 0.05$). Cu-NPs showed anti bacterial activity against *E. coli*.

Key words: Copper nanoparticles, *Escherichia coli*, growth curve

INTRODUCTION

Antibacterial have been widely used in the textile industry, water disinfection, medicine and food packaging. Organic compounds used for disinfection have some drawbacks, including toxicity to the human body, therefore, the interest in inorganic disinfectants such as metal oxide nanoparticles (NPs) is increasing (Von Nussbaum *et al.*, 2006). Antibacterial activities of nanoparticles depend on two main factors, including physicochemical properties of NPs and type of bacteria (Witte, 2004). An important factor that can influence the tolerance of bacteria against NPs is the rate of bacterial growth. Fast growing bacteria are more susceptible to NPs than slow-growing bacteria (Brown *et al.*, 1988). It is possible that the tolerance property of slow-growing bacteria is related to the expression of stress-response genes (Lu *et al.*, 2009). Therefore, antibacterial effects highly depend on the particular strain. The exact mechanisms of NPs toxicity against various bacteria are not fully understood. It is believed that NPs are able to attach to the membrane of bacteria by electrostatic interaction and disrupt the integrity of the bacterial membrane (Thill *et al.*, 2006). The toxicity of NPs depends on the combination of several factors such as temperature, aeration, pH, concentration of NPs and concentration of bacteria. The high temperature, high aeration and low pH decrease the agglomeration and increase the toxicity (Pramanik *et al.*, 2012). In this study, synthesis of copper nanoparticles was approached by a single-precursor route via controlling the growth temperature and Cu-NPs effects on the growth rate of *E. coli* were investigated.

MATERIALS AND METHODS

Preparation of copper nanoparticles: Copper acetylacetonate ($\text{Cu}(\text{AcAc})_2$) with 99.99% purity and oleylamine (OA, 90%) were purchased from Aldrich. In this study, synthesis of copper nanoparticles was approached by a single-precursor route via controlling the growth temperature. In a typical synthesis, about 0.01 mol $\text{Cu}(\text{acac})_2$ was added to the three test flask, which contained 5 mL oleylamine (OA) under Ar while, the temperature kept at 200°C. This temperature kept at the selected value for 5 h. The solutions, then, were cooled at room temperature and nanoparticles were obtained by centrifugation (5 min) and washing. The as-prepared nanoparticles were dried in vacuum at 60°C for 24 h for further experiments.

Determining the growth curves of *E. coli* bacterial cells exposed to different concentrations of Cu nanoparticles: *E. coli* bacterial strain was firstly cultivated in a sterilized nutrient broth at 37°C in a shaker for 24 h. To examine the growth curves of bacterial cells exposed to Cu nanoparticles, nutrient broth with different concentrations of Cu nanoparticles powder (0, 50, 100 and 150 $\mu\text{g mL}^{-1}$) was used. Each culture was incubated in a shaking incubator at 37°C for 24 h. Growth curves of bacterial cell cultures were attained through repeated measures of the Optical Density (OD) at 600 nm. A control medium, which contained no copper nanoparticles, was also used to examine the true differences between samples and control groups.

RESULTS AND DISCUSSION

Transmission Electron Microscopy (TEM): The shape and size distribution of Cu nanoparticles were characterized by Transmission Electron Microscopy (TEM) with a size range between 50-100 nm which indicates that nanoparticles are large and widely dispersed (Fig. 1).

Scanning Electron Microscopy (SEM): The morphology of Cu nanoparticles was examined by SEM. The typical SEM image in Fig. 2 shows that the product mainly consists of particle-like Cu nanoclusters with panoramic view and the size ranges from 40-100 nm. However, further observation with high magnification reveals that these Cu nanoclusters are assembled by smaller nanoparticles, which exhibit good uniformity and the average diameter is about 30 nm. The average size of these nanoparticles is about 20-40 nm, almost in accordance with that from SEM observations. XRD analysis of copper nanoparticles was done by a goniometer. Data was taken for 2θ range of 10 to 80 degrees.

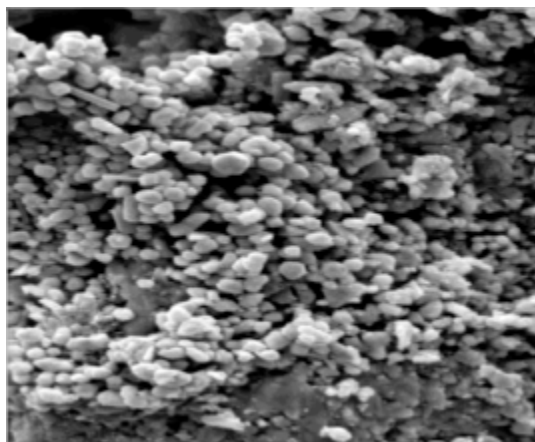


Fig. 1: TEM image of Cu nanoparticles

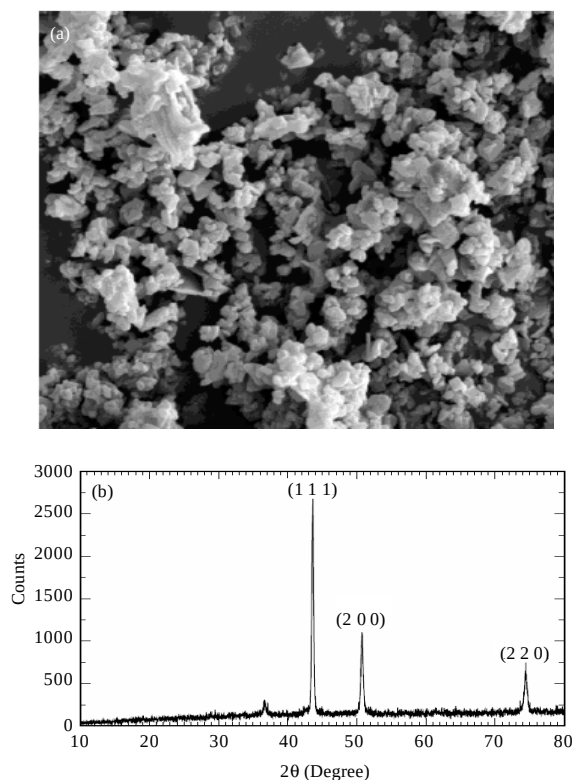


Fig. 2(a-b): (a) SEM image and (b) XRD pattern of Cu nanoparticles

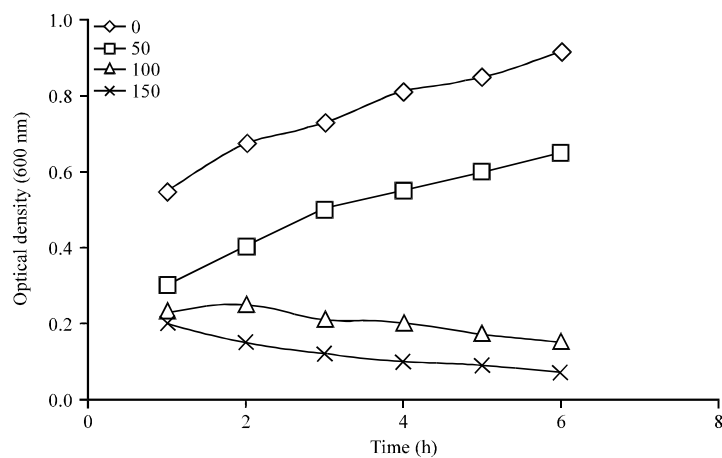


Fig. 3: Growth curves of *E. coli* cells exposed to different concentrations ($\mu\text{g mL}^{-1}$) of Cu-NPs

Growth curves of bacterial cells treated with different concentrations of Cu nanoparticles: The growth curves of bacterial cells treated with Cu nanoparticles indicated that Cu could inhibit the growth and reproduction of bacterial cells. The growth curves is shown in Fig. 3. The bacterial growths of cells treated with 50, 100 and 150 $\mu\text{g mL}^{-1}$ were inhibited. After 2 h, almost all treated bacterial cells were dead. The bacterial growth of the cells treated with 50 $\mu\text{g mL}^{-1}$ Cu nanoparticles was also lower than that of cells in the control group. In general, the

Table 1: Statistical analysis of different concentration of Cu-NPs on *E. coli* growth inhibition

Statistical analysis	Optical density	Concentration
Descriptive statistics		
N	12	12
Mean	0.3788	75.0000
Std. deviation	0.18784	58.38742
Minimum	0.19	0.00
Maximum	0.70	150.00
Test statistics (a, b)		
Chi-Square	10.421	
df	3	
Asymp. Sig.	0.015	

a: Kruskal Wallis Test, b: Grouping Variable, concentration

Kruskal-Wallis ANOVA test showed that there is difference ($p < 0.05$) in microbiological death between various concentration of NPs used (Table 1). In another study conducted for the investigation of the impacts of other nanoparticles on *E. coli*, the *E. coli* cells of control group were typically rod-shaped. Each cell size was almost same and damage on the cell surface was not detected (Danileczuk *et al.*, 2006; Sondi and Salopek-Sondi, 2004).

CONCLUSION

Synthesis of copper nanoparticles was approached by a single precursor route via controlling the growth temperature. The prepared Cu nanoparticles showed efficient anti-bacterial activity against *E. coli*. The results indicated that after 2 h of incubation, almost all treated bacteria cells were dead with $150 \mu\text{g mL}^{-1}$ Cu nanoparticles. Kruskal-Wallis test was used and showed significance differences ($p < 0.05$) between inhibition of growth cells by different amount of Cu-NPs.

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