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Facile Synthesis of ZnO-Cu_2O Composite Nanoparticles and Effect of Cu_2O Doping in ZnO on Antimicrobial Activity

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Abstract

The comparative antibacterial activity of ZnO and ZnO-Cu_2O composite nanoparticles has been investigated and presented in this manuscript. The present study describes the facile synthesis of pristine ZnO nanoparticles and ZnO-Cu_2O composite nanoparticles respectively. ZnO nanoparticles and ZnO-Cu_2O composite nanoparticles have been synthesized by simple solution process using zinc nitrate hexahydrate and copper acetate as sole precursor. The diameter of each ZnO and ZnO-Cu_2O composite nanoparticles lies between 200 to 600 nm respectively as observed from SEM images. Herein, we scrutinized the influence of Cu_2O doping in ZnO on the antibacterial activity. The *E. coli* bacteria which are generally ever-present have been chosen as the model organisms for relative study. The experimental procedures for the antibacterial test include spectroscopic method, taking different concentrations (10–40 µg/ml) of samples to unearth the minimum inhibitory concentration. Our analysis says that the minimum concentration of ZnO and ZnO-Cu_2O composite nanoparticles which inhibited the growth of *E. coli* bacteria was found to be 10 µg. A mechanism was also proposed to describe the better antibacterial activity of the ZnO-Cu_2O composite nanoparticles than the pure ZnO nanoparticles.

Keywords Nanomaterials; Composite; ZnO-Cu_2O; Antimicrobial activity

Introduction

In recent times owing to advancement of some resistant bacterial strains to counter the antibiotics, the antibacterial activity of nanomaterials, such as zinc, silver and copper, with their unique size dependent properties has attracted great attentions [1-5]. For instance, ZnO with a quartzite hexagonal phase have a direct band gap of 3.37 eV possesses a wide range of technological applications. Zinc oxide has attracted wide interest because of its good photocatalytic activity, high stability, non-toxicity and antibacterial property [6-9]. Besides, ZnO particles are effective for inhibiting both Gram positive and Gram-negative bacteria. They also show antibacterial property for spores that are high-temperature resistant and high-pressure resistant [10].

Similarly, copper and its complexes have been widely utilized as effective materials for sterilizing liquids, textiles and also human tissues for centuries [11,12]. Instead, antibacterial activity of copper and copper oxide nanomaterials have limited research, likely due to fast oxidation of metallic copper nanoparticles in exposure to air and both chemical and physical instability of the copper oxides formed at temperature below 200 °C, particularly if Cu_2^+ is formed [13,14]. Also, it has been observed that the composites of metal oxides show superior characteristics than that of single one because of the collective behavior of individual entity [15,16]. This is one of the fast-emerging areas of research. Metal nanoparticles have been widely investigated because of their exceptional physical and chemical properties in contrast to their bulk counterparts. Metal nanoparticles have been extensively researched because of their wide range of applications [17-19]. Keeping in consideration the novel characteristics of composite nanomaterials, in this study, ZnO-Cu_2O composite nanoparticles have been synthesized by simple solution process with high antibacterial activities. The synthesized ZnO-Cu_2O nanoparticles displayed outstanding activity which may have potential application as upcoming antimicrobial materials in the medical and food packaging fields.

Experimental

Materials and reagents

Zinc Nitrate hexahydrate, $Zn(NO_3)_2.6H_2O$ (99.5%), Copper Acetate (99.9%) and hydrazine (98%) were purchased from Sigma-Aldrich. Methanol (analytical grade; Showa Chemicals Ltd., Japan) was used as solvents without further purification. For examining antibacterial property bacterial strain *Escherichia coli* KCCM 11234 was purchased from Korean Culture Centre of Microorganisms (KCCM). Trypton soy broth (Torlak, Belgrade; BD Diagnostic, Becton, Dickinson & Co., USA) was used as growth medium.

ZnO nanoparticles synthesis

2.97 g of zinc nitrate hexahydrate was dissolved in 100 mL distilled water and the mixture was stirred for sometimes until the mixture became as a homogeneous, then this mixture was heated at 50°C. Little amount of 2 M of NaOH was added dropwise to the solution, white precipitate was obtained. The obtained wet sample was filtered few times with water and then with methanol. Finally, the result was washed by methanol several times followed by washing with distilled water. The obtained white powder was oven dried at 80°C and calcined at 200°C for 2 hours.

ZnO-Cu_2O composite nanoparticles synthesis

1 mole of cupric acetate was dissolved in 50 ml of distilled water in a beaker and stirred at 50°C. Few drops of 2 M of NaOH were added to the solution while stirring, light blue precipitate was formed. Next, 1 ml of hydrazine solution (85 wt%) was added to the solution. After some time, the blue color of precipitate changed to the orange brown. In a separate beaker, 1 mole of zinc nitrate was dissolved in 50 ml of distilled water and stirred. Some drops of 1 M of NaOH added while stirring, white precipitate was obtained. Then, we mixed the solution from both the beaker and the resulting solution was kept stirring for 30 minutes. The precipitant was filtered off, washed several times by methanol and water. Finally, the obtained powder was oven dried at 80°C and calcined at 200°C for 2 hours.

Instruments and conditions

The X-ray diffraction (XRD) patterns of samples were recorded on a Rigaku/Max-3A X-ray diffractometer with CuK radiation (λ=1.5418 Å) and the operating voltage and current were maintained at 30 kV and 40 mA, respectively. To examine the microstructure, the images were observed by a scanning electron microscope (SEM, S-7400, Hitachi high technologies, Japan). The Thermos-Gravimetric Analysis (TGA) curves of pure ZnO and ZnO-Cu_2O composite was carried out by using thermo gravimetric analyzer (Perkin–Elmer Inc., TGA 6, USA). Measurements were conducted over a temperature range of 40-700°C at a heating rate of 10°C/min in nitrogen atmosphere.

Antibacterial activity of ZnO and ZnO-Cu_2O composite nanoparticles

Amna et al. methodology with some appropriate changes has been adapted in the present study to evaluate the antibacterial activity of pristine ZnO and ZnO-Cu_2O composite nanoparticles against the food pathogen *E. coli* [20]. To facilitate antibacterial activity test of our synthesized materials (ZnO and ZnO-Cu_2O composite), the *E. coli* strain was primarily developed on solid medium and overnight grown colonies from agar plates have been inoculated into broth (100 ml). The seed preparation was done in the Trypton soy broth with 0.6% yeast extract and pH of broth was maintained at 7.3. The cultures were incubated at 37°C. The culture expansion was checked at every 4 h by UV-Vis spectrophotometer. To solve the purpose of testing out the minimum inhibitory concentration of synthesized pristine and composite nanoparticles, different concentrations (0, 10, 20 and 40 μg/ml) of ZnO and ZnO-Cu_2O have been added in 100 ml of freshly prepared broth. The treated cultures were further incubated at 37°C in a rotary shaker with shaking at 150 rpm. Uninoculated and an inoculated control devoid of ZnO and ZnO-Cu_2O composite nanoparticles were also set aside. The bacterial turbidity was checked by taking optical density (OD) with absorbance at 600 nm by UV-spectrophotometer at an interval of 4 h for 20 h.

Results and Discussion

The phase structure of synthesized samples was characterized by XRD and results were shown in Figure 1. The diffraction peaks of pure ZnO nanoparticles could be indexed to the hexagonal phase (JCPDS Card No. 36–1451) as shown in Figure 1a. Whereas in Figure 1b, besides the diffraction peaks from ZnO, all other peaks can be indexed to a single phase of crystalline cubic Cu_2O (JCPDS No. 78-2076) without an absence of other crystalline forms [21]. Hence, it can be deduced that, in the composite sample, the ZnO and Cu_2O phase coexist. The typical SEM images of pure and composite nanomaterial were prepared after calcination at 200°C is shown Figures 2a and 2b. The microstructure of pure ZnO is showing non-uniform nanoparticles having size in the range of 300-600 nm (Figure 2a). Whereas, composite nanoparticles are composed of homogeneous and uniform nanoparticles having grains size in the range of 200-300 nm (Figure 2b).

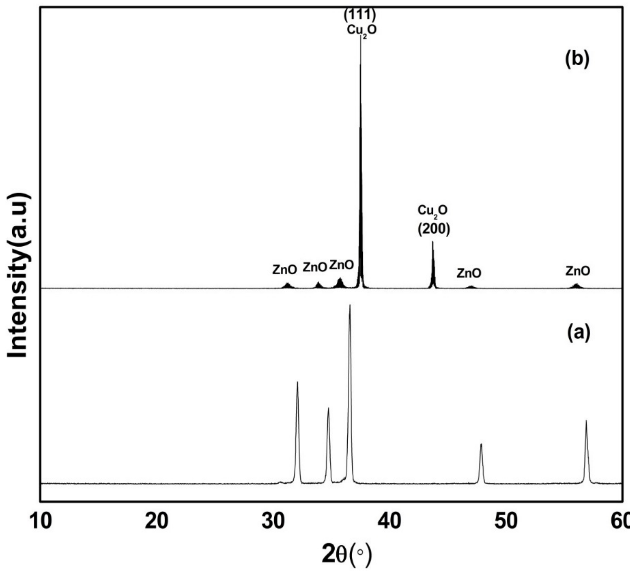

Figure 1: XRD pattern of synthesized (a) pristine ZnO and (b) composite ZnO-Cu_2O nanoparticles.

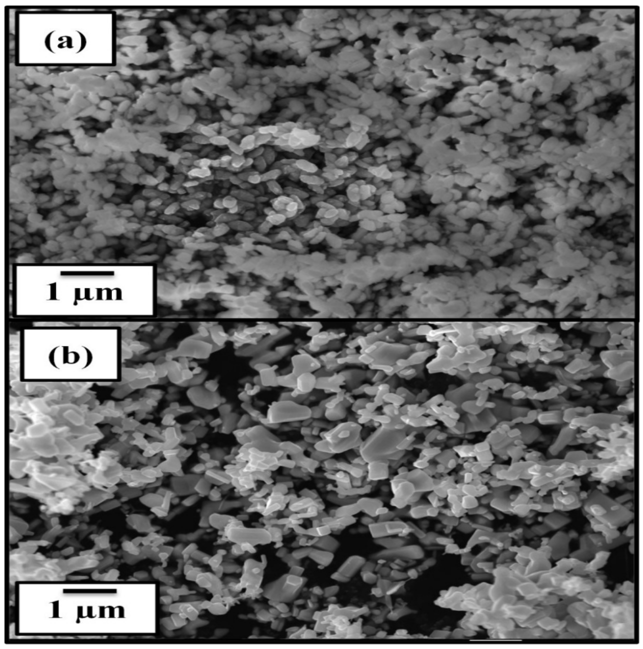

Figure 2: SEM image of synthesized (a) pristine ZnO and (b) composite ZnO-Cu_2O nanoparticles.

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The TGA thermograms of pristine ZnO and ZnO-Cu_2O composite nanoparticles were shown in Figure 3. As pointed out by the TGA, pure ZnO nanoparticles decompose up to 7.35% while ZnO-Cu_2O composite nanoparticles shows 7.11% degradation in the temperature range of 40-700°C. As pointed out by the TGA graph, the pure ZnO decomposed up to 93.04% between 40 and 38°C. On the other hand, ZnO-Cu_2O composite degraded up to 93.04% between 40 and 385°C, Both ZnO-Cu_2O composite and ZnO showed sharp weight loss between 50 and 280°C due to the loss of water, nitrate and acetate molecules from zinc nitrate and copper acetate respectively. The graph shows that ZnO-Cu_2O composite have relatively higher thermal stability than pure ZnO which may be due to the interaction of copper and zinc oxide.

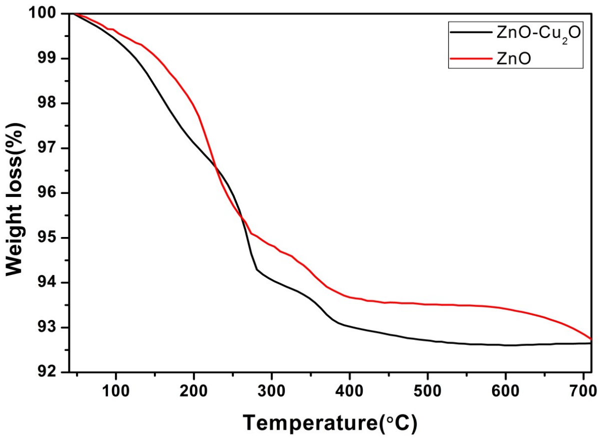

Figure 3: TGA graphs of synthesized (a) pristine ZnO and (b) composite ZnO-Cu_2O nanoparticles, under nitrogen atmosphere.

The growth rates of control *E. coli* (EC) culture and the cultures disinfected with pure ZnO and ZnO-Cu_2O composite nanoparticles have been shown in Figures 4a and 4b. The utilized *E. coli* culture when treated with different concentrations (0, 10, 20 and 40 µg/ml) of above mentioned pure and doped nanoparticles, illustrated growth curves which comprise of different phases such as the lag phase, exponential phase and decline phase. However, decline phases in each growth curve could not be known as we only analyzed the total numbers of bacteria based on the value of OD600. In the absence of pure ZnO and ZnO-Cu_2O composite nanoparticles, *E. coli* grew exponentially. But when exposed to abovementioned concentrations of pure ZnO and ZnO-Cu_2O composite nanoparticles, *E. coli* cells showed interruption in the exponential growth and with the increase in concentration of pure ZnO and ZnO-Cu_2O composite nanoparticles, the delay in growth was incredibly apparent. It has been observed that the lowest concentration of the pure ZnO and ZnO-Cu_2O composite nanoparticles that inhibits growth of microbial strain was found to be 10 µg/ml. Furthermore, it has been seen that with amplification in concentration of pure ZnO and ZnO-Cu_2O composite nanoparticles solution, inhibition has also increased. Very clear difference in the growth rate has been observed after 4 h of incubation with pure ZnO and ZnO-Cu_2O composite nanoparticles. The highest concentration (40 µg/ml) of ZnO-Cu_2O composite nanoparticles has demonstrated to excellent inhibition of *E. coli.* We hypothesize that the increased growth inhibition and disinfection by ZnO-Cu_2O composite nanoparticles may be due to the synergistic effect of ZnO and Cu_2O nanoparticles. The predictable mechanism to be assumed is that when we disperse ZnO and ZnO-Cu_2O in the growth media, the Zn^{+2} and Cu^{+2} atoms present in ZnO and ZnO-Cu_2O interacted with the bacterial cells and adhered to *E. coli* cell wall. The overall charge on bacterial cell surface at biological pH is negative, which is due to the high number of carboxylic and other groups which on dissociation make the cell surface negative [22]. Therefore, bacteria and Zn^{+2} and Cu^{+2} atoms own different charges and these electrostatic forces may be the reason for their adhesion and bioactivity. As well, the production of reactive oxygen species (ROS) by CuO nanoparticles in aqueous suspensions also occurred. Usually electrons reduce O_2 to produce superoxide anion $O_2^{-\bullet}$. ROS generated in liquid culture interacted with wall of the cell and these generated free radicals go inside of the cell leading to disruption of the internal cell organelles and as a result the death of *E. coli.*

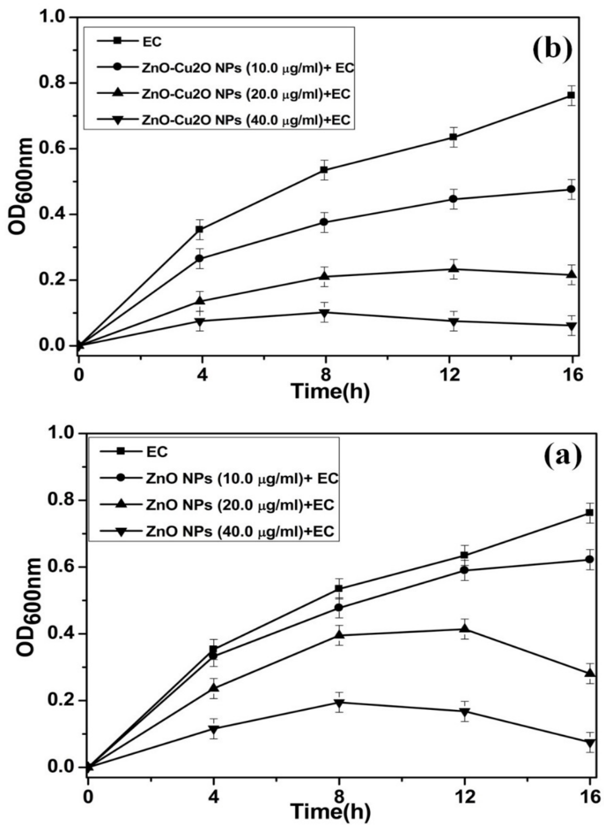

Figure 4: Growth curve of *E. coli* cells exposed to different concentrations of (a) pristine ZnO and (b) composite ZnO-Cu_2O nanoparticles.

Conclusion

In summary, ZnO-Cu_2O composite nanoparticles have been successfully prepared via non-hydrolytic solution. The work presents the groundwork studies on the antibacterial activities of the pure ZnO and ZnO-Cu_2O with different concentrations and storage periods. The results have demonstrated that the culture which was disinfected with ZnO-Cu_2O nanoparticles, which were stored for 16 h, had the best antibacterial behavior against *E. coli* bacteria. The better antibacterial activities were believed to be caused by the photocatalytic properties of ZnO and ZnO-Cu_2O particles, there is an interaction between the bacteria cells and the ZnO-Cu_2O particles, which is presumably due to the electrostatic forces. The test of antimicrobial activities showed that ZnO-Cu_2O composite nanopaticles had higher antimicrobial activities than pure ZnO nanopatcles, indicating that the composite of Cu_2O and ZnO enhanced the antimicrobial activities of ZnO-Cu_2O nanoparticles due to synergistic effect. On the whole, the preliminary findings suggest that ZnO-Cu_2O nanoparticles can be used externally to control the spreading of bacterial infections. In the prevention and control of bacterial spreading and infections, the main target is the cell wall structure.

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