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Effect and Properties of Surface-Modified Copper Doped ZnO Nanoparticles (Cu:ZnO NPs) on Killing Curves of Bacterial Pathogens

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ABSTRACT

Background: The current study aimed to determine the effect and properties of surface-modified copper doped Cu:ZnO NPs on killing curves of bacterial pathogens.

Methods: Preparation of *in situ* surface-modified copper doped ZnO nanoparticles (Cu:ZnO NPs) was done according to standard procedure. Assay for antimicrobial activity of Cu:ZnO NPs against bacterial pathogens was carried out based on disc diffusion method. Determination of shelf life, thermal and pH stability of antibacterial activity of Cu:ZnO NPs was done and residual activity was determined against the target cultures.

Results: FTIR spectra indicate that the nanomaterials synthesized have higher peak intensity compared with reagent grade ZnO. According to the SEM image the nanoparticles synthesized have different size and heterogeneous morphology. 400 ppm of Cu:ZnO NPs gave zones of inhibition with diameters of 9.0 – 16 mm against the target cultures. Amongst the target cultures, *Escherchia coli* was the most sensitive to the Cu:ZnO NPs inhibition zone diameter 16 mm; whereas, 9 mm wide inhibition zone was obtained against *Staphylococcus aureus*. The Cu:ZnO NPs was fairly stable for a period of 60 days at room temperature (RT) showing lost of only 20% and 30% antibacterial activity as tested against *E. coli* and *S. aureus*, respectively. The Cu:ZnO NPs was quite stable at this pH and temperature range tested against both *E. coli* and *S. aureus*.

Conclusion: Surface-modified copper doped Cu:ZnO NPs have significant potential for their usefulness as antibacterial agents.

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Introduction

Despite advances in antimicrobial therapies, the re-emergence of infectious diseases and the continuous development of antibiotic resistance among disease-causing bacteria pose a serious threat to public health all over the world (1). Within the past decades, a wide variety of antibacterial substances have been exposed, designed and synthesized and finally some of them have been successfully used for controlling infectious diseases (2).

These agents can be classified based on various topics; for example organic and inorganic. Organic antimicrobial agents are often less stable particularly at high temperatures (3). This presents a potential problem for the new product. As a consequence, inorganic materials such as metals like Zn have attracted lots of attention over the past decade due to their ability to withstand harsh process conditions (4).

Furthermore, recent advances in the field of nanotechnology, particularly the ability to prepare highly ordered nanoparticles of any size and shape, have led to the development of new biocidal agents (5). Several studies have indicated that nano particulate formulations can be used as effective bactericidal materials (6-8).

Thus, zinc oxide (ZnO) and copper oxide nanomaterials are incorporated into a variety of medical and skin coatings because of their antimicrobial and / or antifungal properties; and indeed they are generally regarded as safe materials for human beings and animals (8, 9).

Jiang *et al*. reported that ZnO nanoparticles were the most toxic antibacterial agent among the other examined metal oxide nanoparticles like aluminum, silicon and titanium against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas fluorescens* (10). Therefore, the current study aimed to determine the effect and properties of surface-modified copper doped Cu:ZnO NPs.

Materials and Methods

Preparation of in situ surface-modified copper doped ZnO nanoparticles (Cu:ZnO NPs)

Preparation of *in situ* surface-modified copper doped ZnO nanoparticles (Cu:ZnO NPs) was done according to standard procedure (11).

Cu:ZnO NPs were fabricated under mild hydrothermal conditions (T = 100°C, P = autogenous, t = 12 hr). 2 mole of pure ZnO (Merck, Germany) was taken as starting material and the dopant, copper oxide (Merck, AG, 99%) at 0.5, 1, 1.5, 2, and 2.5 mol% was added into it. A certain amount of 1 M NaOH (Merck, AG) was added as mineralize to the precursors. At the same time, 1 ml of *n*-butylamine (Merck, LG) was added to the above-mentioned mixture and it was stirred vigorously for a few minutes. The final compound was then transferred to the Teflon liner (Vfill = 10 ml), which was later placed inside a General Purpose autoclave. Then the assembled autoclave was kept in an oven with a temperature programmer-controller for 12 h. The temperature was kept at 100°C. After the experimental run, the autoclave was quenched to the room temperature. The product in the Teflon liner was then transferred to a clean beaker, washed with double distilled water several times, and then allowed to settle down. The surplus solution was removed using a

syringe. Finally, the remnants were allowed to dry naturally at room temperature. The dried nanoparticles were subjected to systematic characterization and antimicrobial studies. The synthesized nanoparticles were characterized using Fourier transform infrared spectroscopy (Tensor 27 spectrophotometer, Bruker Optic, GmbH, Germany) and scanning electron microscopy (SEM, JOEL. Ltd., Tokyo, Japan).

Bacterial Strains

Pseudomonas aeruginosa, *E. coli*, and *Staphylococcus aureus* were examined for their susceptibility toward the treatment with Cu:ZnO NPs. These multidrug resistant-strains were maintained at 4^{o}C on nutrient agar slants containing 5% glycerol.

Assay for antimicrobial activity of Cu:ZnO NPs against bacterial pathogens

Bacterial suspensions of freshly grown cultures were prepared in sterile saline and adjusted to a density of 10^{7} cells/ml. Cu:ZnO NPs was serially diluted and five discs were soaked in 50 µl of each dilution. Discs containing Cu:ZnO NPs at different concentrations were placed on the agar plates and incubated overnight. Experiments were carried in duplicate.

Determination of shelf life

The Cu:ZnO NPs were stored at room temperature and refrigerator for 60 days. At different time intervals 50 µl was removed and residual activity was determined weekly against the target cultures.

Thermal stability of antibacterial activity of Cu:ZnO NPs

To determine the effect of temperature on stability of Cu:ZnO NPs, screw ampoules containing 100 µl of ZnO:Cu were kept at 40, 50, 60, and 70 mg/l for one hour in water bath and then residual activity was determined against the target cultures.

Effect of pH on the antibacterial activity of essential oil

100 µl of Cu:ZnO NPs was mixed with 100 µl of phosphate buffer of different pH, incubated for one hour and then residual activity was determined against the target cultures.

Effect of Cu:ZnO NPs on growth of target cultures

Effect of Cu:ZnO NPs on growth of target cultures was determined. Briefly, each target culture was inoculated separately in 10 ml of TSB containing 400 ppm of Cu:ZnO NPs. Every one hour 100 µl of sample was withdrawn, for determination of total viable counts (TVC). Each target culture inoculated in 10 ml of TSB served as control showing the normal growth.

Results

FTIR spectra indicate that the nanomaterials synthesized have higher peak intensity compared with reagent grade ZnO *(Figure 1)*. However, no new peaks were observed. According to the SEM image indicates that the nanoparticles synthesized have different size and heterogeneous morphology; among them nanorods and spherical nanoparticles are more obvious (*Figure 2*). In some parts agglomeration has occurred, which are attributed to the inherent

nature of the nanoparticles synthesized. Moreover, the change in the morphology could be contributed to the effect of dopant and surface modifier applied.

As seen in Table 1 400 ppm of Cu:ZnO NPs gave zones of inhibition with diameters of 9.0-16 mm against the target cultures. Amongst the target cultures, *E. coli* was the most sensitive to the Cu:ZnO NPs inhibition zone diameter 16 mm; whereas, 9 mm wide inhibition zone was obtained against *S. aureus*.

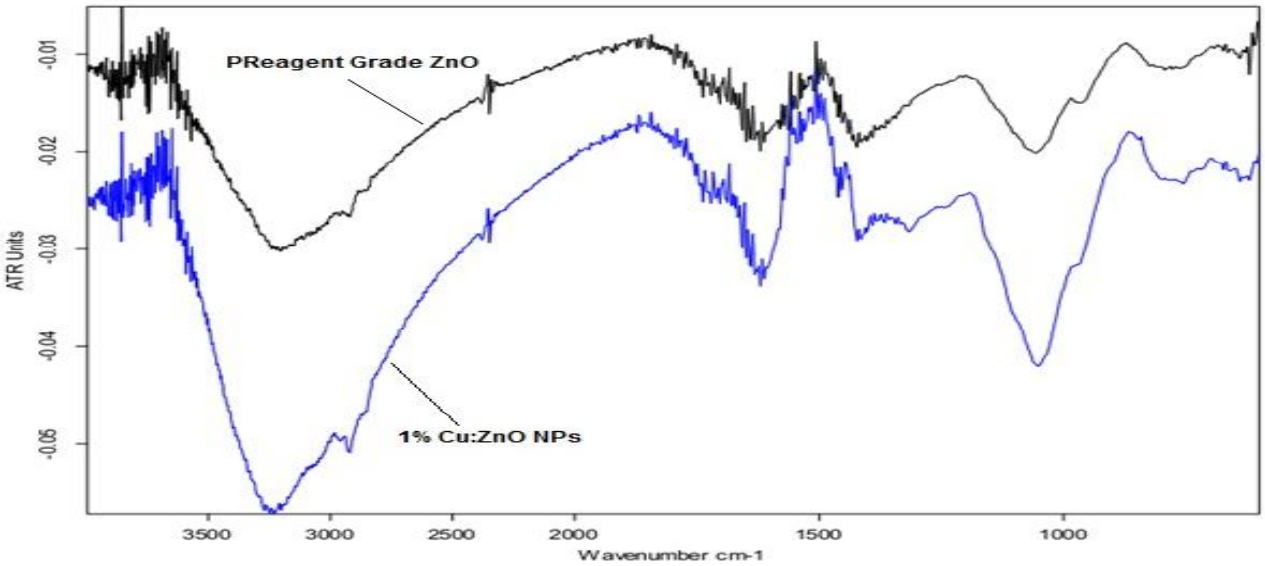

Figure 1. FTIR Spectra of the Cu:ZnO NPs

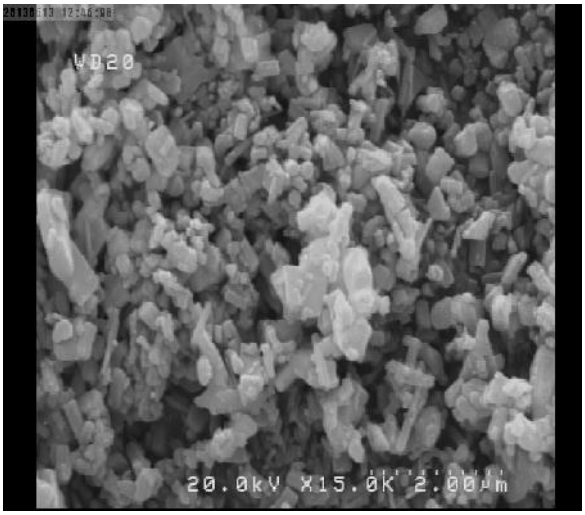

Figure 2. Characteristic SEM image of Cu:ZnO NPs

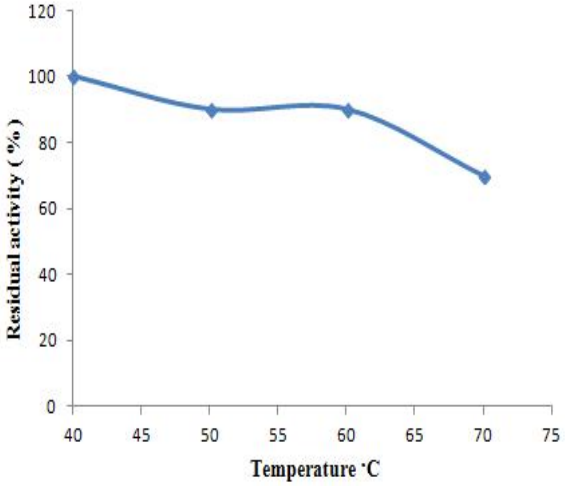

Figure 3. Thermal stability of Cu:ZnO NPs

The Cu:ZnO NPs was fairly stable for a period of 60 days at room temperature (RT) showing lost of only 20% and 30% antibacterial activity as tested against *E. coli* and *S. aureus* respectively (data not shown). Since the activity of the Cu:ZnO NPs was quite stable at RT, experiments were also conducted to see the effect of elevated temperature on stability of Cu:ZnO NPs. As seen in Figure 3 heat inactivation curve, the loss in activity upon heating was relatively gradual in both the cases. Similarly, stability of Cu:ZnO NPs at different pH was checked by pre-incubating the ZnO:Cu for one hour, in phosphate buffer at different pH ranging from 5-9. The Cu:ZnO NPs was quite stable at this pH range tested against both *E. coli* and *S. aureus*.

To determine the effect of Cu:ZnO NPs on growth of target cultures, 400 ppm of Cu:ZnO NPs was added to TSB which showed significant potential antibacterial activity (*Figure 4*).

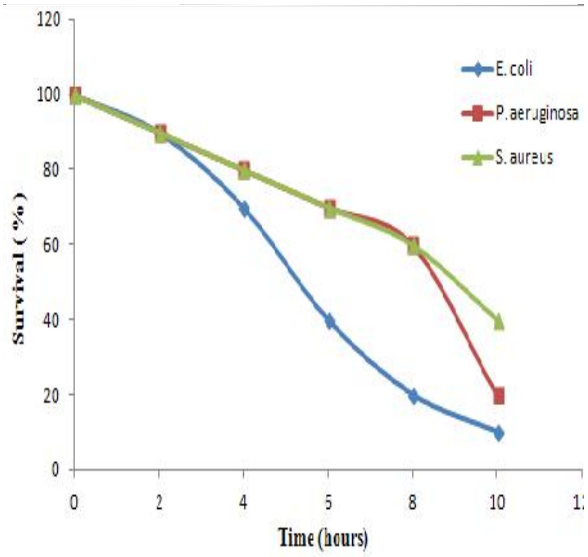

Figure 4. Effect of Cu:ZnO NPs on growth of the target cultures

Discussion

As the microorganisms have developed resistance to most of the antibiotics, the research for novel antibiotic utmost important and for this reason, the present work was carried out to find out the effect and properties of Cu:ZnO NPs on killing curves of bacterial pathogens. Cu:ZnO NPs demonstrated good antibacterial activity against the target cultures. Many scientists from all over the world reported that metal oxide nanomaterials increased cell death with increasing concentration (11-14). Overall, nanotechnology involves the design of materials at atomic point to achieve distinctive properties, which can be suitably manipulated for the desired applications (15).

Our results with a simple approach clearly showed to have antimicrobial effects, proved the inhibition of human pathogens as shown in Table 1; and its bactericidal property was concentration depended. Therefore, here we can propose Cu:ZnO NPs as an ingredient for the dermatological applications in creams, lotions and ointments on account of its antibacterial properties as many scientists also reported the same proposed (16). Although many scientists believe that some nanoparticles like ZnO are known to be toxic human, but our results showed at relatively low concentration showed a good activity against the bacterial pathogens; this hypothesis is in agreement with many other reports on nano particles (5, 17). In fact, it has been shown that ZnO protects against intestinal diseases by protecting intestinal cells from *E. coli* (ETEC) infection by inhibiting the adhesion and internalization of bacteria (18).

Table 1. Antibacterial activity of Cu:ZnO NPs

Target culture	Concentration of Cu:ZnO NPs		
	400 ppm	200 ppm	100 ppm
	Inhibition zone diameter (mm)		
Escherishia coli	16	13	10
Pseudomonas aeruginosa	13	10	7
Staphylococcus aureus	09	07	4

Conclusion

The antibacterial activity was quite stable at room temperature as well as to various temperatures. We also studied the effect of pH on the residual activity of the Cu:ZnO NPs which did not abolished its effect on the target cultures. A potential use of Cu:ZnO NPs need more studies particularly evaluation of its toxicity.

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Conflict of Interest

None declared conflicts of interest.

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