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ANTIBACTERIAL AND ANTIBIOFILM EFFICACY OF COPPER DOPED ZnO NANOPARTICLES AGAINST MARINE BIOFILM FORMING BACTERIA

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ABSTRACT

The antibacterial and antibiofilm activity of chemically synthesized pure and Cu doped ZnO nanoparticles against marine biofilm forming bacteria has been reported for the first time. The synthesized nanoparticles were characterized using PXRD, FTIR and SEM which confirmed the formation of phase pure nanostructured zinc oxides. The antibacterial and antibiofilm activities of the synthesized nanoparticles were tested against five marine bacterial isolates (MB1, MB2, MB3, MB4, and MB5), which revealed the enhanced activity of Cu doped ZnO with increasing content of the doping agent. The zone of inhibition and the percentage of biofilm inhibition were found to be the highest (9 mm and 94% respectively) for the isolate MB5 indicating the potential of the Cu doped ZnO as an effective antifouling agent.

KEYWORDS: **Nanoparticles, FTIR, Marine biofilm, Antibacterial activity, Microtiter plate**

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INTRODUCTION

Synthesis of semiconductor nanoparticles is gaining more interest in recent years due to their interesting physical and chemical properties. Among the metal oxide nanoparticles ZnO is an attractive and promising material due to its wide band gap (3.37 eV at room temperature), large exciton binding energy (60 meV), n-type conductivity, abundance in nature and eco-friendly property. These characteristics make this material attractive for many applications, such as solar cells, optical coatings, photo catalysts, electrical devices, antibacterial coatings and in gas sensors [1, 2]. Properties of ZnO can be modified according to our application, by doping with various metal atoms. The metal doping induces drastic changes in optical, electrical and magnetic properties of ZnO by altering its electronic structure which is important for its practical applications e.g., doping with aluminum to improve solar cells [3], with copper for higher sensitivity to certain gases [4], and with manganese to endow it with antibacterial activity [5]. Biofilm formation has serious implications in industrial, environmental, public health, medical situations [6]. The biofilm forming micro-organisms have advantages in that they are more difficult to mechanically remove from surfaces and are more resistant to disinfectants compared with planktonic forms [7, 8]. Therefore the development of nanoparticles with antimicrobial properties is of considerable interest. Several studies have reported the effect of nanoparticles on biofilm forming bacteria [9, 10]. To our knowledge, there is no investigation reported in the literature on the antibacterial and antibiofilm activities of Cu-doped ZnO nanoparticles. In the present study the antibacterial activity of Cu-doped ZnO nanoparticles and its effect on marine biofilm formation has been investigated and reported for the first time.

MATERIALS AND METHODS

Preparation of pure and Cu-doped ZnO nanoparticles

Pure and Cu-doped Zno nanoparticles were synthesized according to the method previously described by Ghobti et al [11]. To synthesize pure ZnO nanoparticles, 0.2 M Zn $(NO_3)_2$ solution was prepared to which 0.5 M NaOH was added dropwise under vigorous stirring and pH 7.0 ± 0.05. Similarly Cu doped ZnO was prepared by dropwise addition of 0.5 M NaOH to 0.2 M Zn $(NO_3)_2$ containing various molar percentages of copper (2-6 %) under the same conditions. The products were hydrothermally decomposed by treating them at 500°C for 2 hrs in a muffle furnace to obtain pure and copper doped ZnO nanoparticles and they were labeled as ZO, CZO2, CZO4, and CZO6.

Characterization

The crystal structure of the nanoparticles has been investigated through PXRD technique. The XRD patterns were collected on BRUKER-AXSD8 ADVANCE X-ray Diffractometer with CuKα radiation of wavelength 1.541° and scanning angle 2θ over the range of 10°- 80°. The FTIR spectral analysis of the samples were recorded using BRUKER VECTOR 33 spectrophotometer in the range, 400-4000 cm^{-1} in order to confirm the functional groups. The surface morphology of the prepared powders was determined using scanning electron microscope (PhilipsXL 20SEM).

Antibacterial activity

Antibacterial activity of the synthesized nanoparticles were tested against five marine biofilm forming bacteria (MB1, MB2, MB3, MB4, MB5) which were isolated from biofilm samples scraped from ships anchored at Rayapuram harbor, Chennai, Tamil Nadu. Agar well diffusion method was used to study the bactericidal activity of the nanoparticles. Overnight broth culture of each bacterial isolate was adjusted to 0.5 McFarland standards and a lawn culture was made on Mueller Hinton Agar plates. Wells of 8 mm diameter were made using sterile well puncture and 100 µl of each nanoparticle solution (1 mg/ml) was loaded in their respective wells and incubated at 37°C for 24 hrs. The zone of inhibition was calculated by subtracting the well diameter from total inhibition zone diameter.

Antibiofilm assay

Antibiofilm assay was carried out by microtiter plate technique previously described by Mohanty et al [12] with minor modifications. Briefly overnight grown cultures were diluted 1:100 in fresh nutrient broth and inoculated in individual wells of sterile 96-well microtiter plate. The synthesized nanoparticles (1mg/ml) were loaded in each well and incubated at 37°C for 24 hrs. After incubation the wells were washed twice with sterile phosphate buffered saline in order to remove loosely attached cells. Biofilms formed by adherent cells were quantified by recording the optical densities at 575 nm and the percentage of biofilm inhibition was calculated.

RESULTS AND DISCUSSION

PXRD analysis

PXRD patterns of ZO, CZO2, CZO4, and CZO6 (Fig.1) show sharp and intense peaks which imply high crystallinity of the samples. The analysis of diffraction peaks further reveals the wurtzite structure of the material (JCPDS No.70-2551) and also no traces of impurities related to copper phase has be detected indicating that the Cu ions have well substituted Zn sites without affecting its crystal structure. This can be attributed to the fact that ionic radius of Cu^{2+} (0.73A) is very close to that of Zn^{2+}. The mean crystallite sizes have been estimated using Debye-Scherrer equation (1).

$$D = \frac{K\lambda}{\beta cos\theta}$$

where D is the average crystallite size, K is scherrer constant, λ the wavelength of incident X- ray beam, β is the angular width of the diffraction peak at the half maximum in the radian on 2θ scale, θ is the Bragg's diffraction angle.

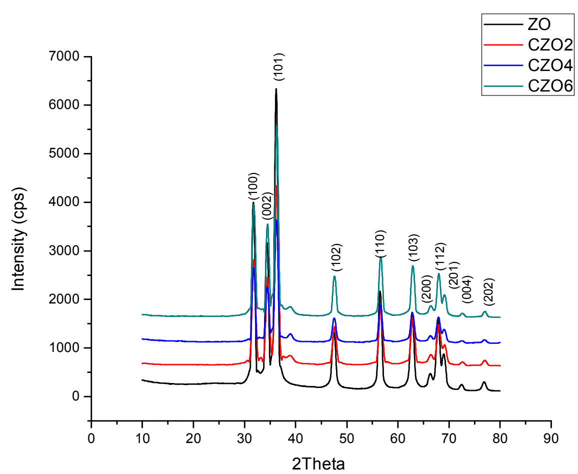

Figure 1
PXRD patterns of pure and Cu doped ZnO nanoparticles.

The average crystallite size of the ZO, CZO2, CZO4 and CZO6 was found to be 32.6, 30.4, 28.3 and 26.5nm respectively. It is seen that compared to pure ZnO, Cu doping in ZnO resulted in decreased crystal size that can be attributed to the formation of Cu-O-Zn on the surface of the doped samples, which hinders the growth of crystals [13].

FTIR and SEM
Fig.2 shows the FTIR spectra of pure and Cu doped ZnO. A high intense band was observed for the pure ZnO at around 413-503 cm^{-1} which corresponds to Zn-O stretching vibration [11]. The broad band at 3444 cm^{-1} and a weak band at 1629 cm^{-1} correspond to the hydroxyl groups of adsorbed water molecules. Two weak peaks at 1076.28 and 1028.06 cm^{-1} and a sharp peak at 1384.89 cm^{-1} are attributed to nitrate groups adsorbed on the surface of zinc oxide in trace amounts [14].

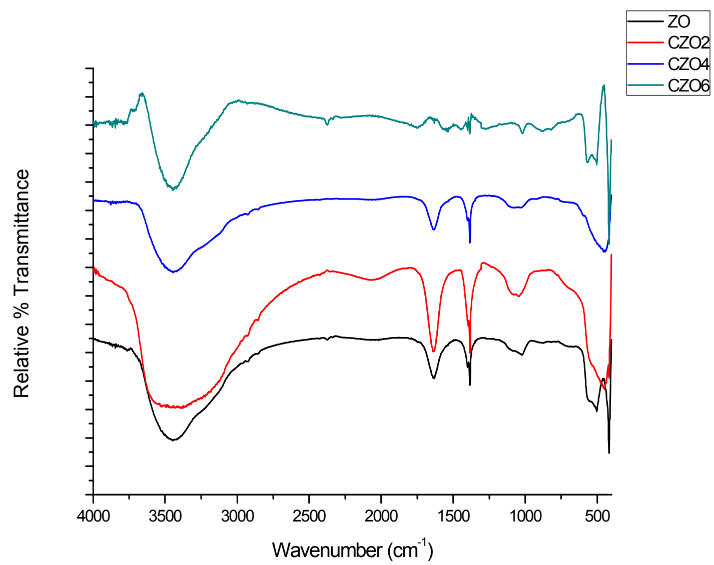

Figure 2
FTIR spectra of undoped and Cu doped ZnO.

Fig. 3(a) and (b) shows the morphology of pure (ZO) and Cu doped Zinc oxide (CZO6). It was observed that the SEM images of the samples revealed that the crystallites are in nanometer size and there is no significant change in morphology of ZnO on doping.

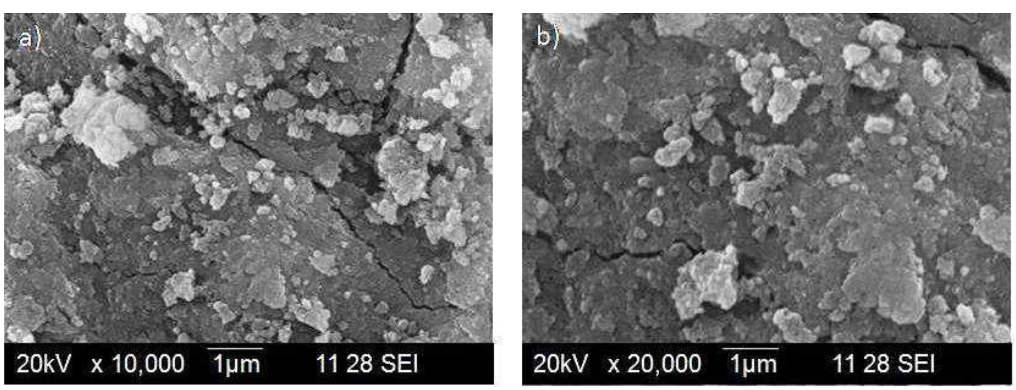

Figure 3
SEM images of (a) ZO (b) CZO6.

Antibacterial activity
Pure and Cu doped ZnO nanoparticles exhibited remarkable antibacterial activity against all the tested bacterial isolates. Among the five tested isolates the antibacterial activity was found to be the highest for the isolate MB5 (Fig. 4). The zone of inhibition for ZO was found to be 3 mm and for CZO2, CZO4 and CZO6 it was 4 mm, 6 mm and 9 mm respectively. The above results indicate that

the antibacterial activity gradually increase with increasing molar percentage of the doping agent, copper (CZO6>CZO4>CZO2>ZO).

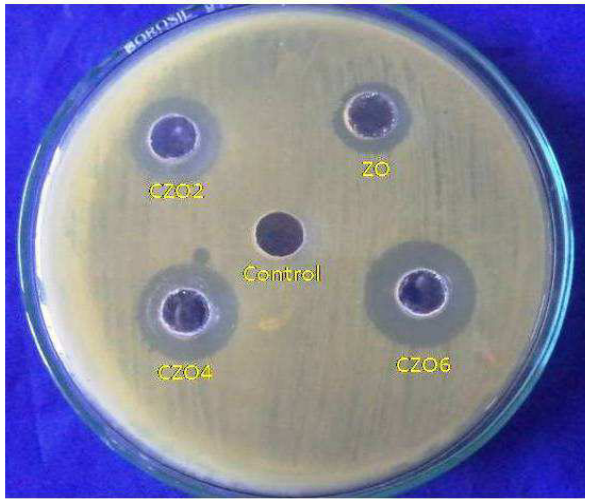

Figure 4
Antibacterial activity Cu doped ZnO against MB5
(a) ZO (b) CZO2 (c) CZO4 (d) CZO6 (e) Control

Antibiofilm assay
Cu doped ZnO nanoparticles were very effective in inhibition of adhesion and biofilm formation against all tested isolates. The percentage of biofilm inhibition was found to the highest (96.2 %) for MB5 compared to the other isolates and it increased with increase in doping concentration (Fig.5). Higher antibacterial and antibiofilm activities of Cu doped ZnO nanoparticles observed in this study can be attributed to their decreased particle size compared to pure ZnO nanoparticles.

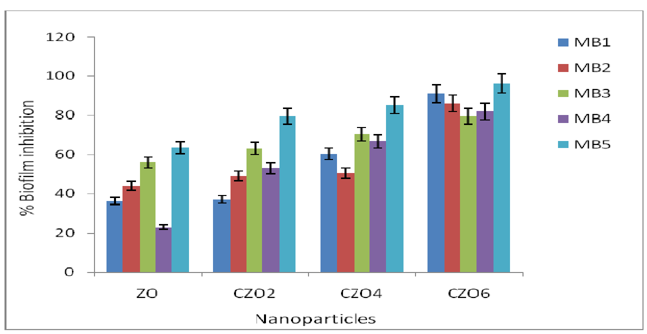

Figure 5
Antibiofilm activity of undoped and Cu doped ZnO (Conc.1mg/ml)

CONCLUSION

Pure and Cu doped ZnO nanoparticles have been synthesized by precipitation method. PXRD studies indicate the formation of phase pure wurzite structure of ZnO. FTIR spectra have shown a broad absorption band around 413-503 cm^{-1} for all the samples which corresponds to the stretching vibration of Zn-O bond. The synthesized pure ZnO nanoparticles showed remarkable antibacterial and antibiofilm activities. These two properties were further enhanced by doping with copper at different molar percentages. Since the antibiofilm activity was proved against marine biofilm forming bacteria the Cu-doped ZnO nanoparticles may be used as a promising antifouling agent to combat marine biofouling.

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