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Research Note

Dopant induced changes in physical properties of ZnO:Mg nanosuspensions: Study of antibacterial activity

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KEYWORDS

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Abstract. The sol-gel method is successfully used for the synthesis of ZnO nanoparticles doped with different concentrations of Mg. The structure, morphology, chemical composition, optical and antibacterial activity of the nanoparticles were studied as a function of the Mg doping concentration. The synthesized ZnO samples show a hexagonal Wurtzite structure, and the phase segregation takes place for the 15% amount of doping. TEM images verify the formation of nanoparticles in the range of 30-55 nm. FTIR and EDS results also confirm the successful incorporation of Mg in the ZnO structure. Optical investigation indicates an increase in band gap energy with an increase in doping content. The antibacterial activities of the nanosuspensions were tested against *Escherichia Coli* (gram negative) and *Streptococcus Mutans* (gram-positive) cultures. ZnO:Mg nanosuspension shows high antibacterial activity, which slightly decreases with the amount of Mg.

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1. Introduction

Zinc oxide has been the subject of a great deal of research in recent years, due to its interesting properties, such as wide band gap energy (3.2 eV), high exciton binding energy (60 meV), and piezoelectricity [1-3]. In addition, the low toxicity and biocompatibility of ZnO have made it a promising candidate for biological and medical applications [5,6]. In the case of antibacterial activities, the use of Zinc oxide has several advantages: it is a mineral element essential to the human body, has strong antibacterial activity in small amounts without

the irradiation of light, and has antibacterial activity in neutral pH [7]. Although the exact mechanism of its antibacterial action is not clearly known, several mechanisms have been proposed: electrostatic interactions, metal ion release, ROS (Reactive Oxygen Species) generation, and membrane damage. Cellular internalization of the nanoparticles has also been reported [7-9].

As particle size reduces to nanometer scale, the physical properties change dramatically. The enhanced bactericidal effectiveness of metal nanoparticles has been suggested to be due to their high surface-to-volume ratio. Such characteristics allow them to interact closely with bacterial membranes, in addition to the release of metal ions. Therefore, nanotechnology, by enabling manipulation of the size and shape of particles, could have a drastic influence on their antibacterial behavior [8]. Doping could also alter the

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bactericidal effect of ZnO, as reported in the work of Yamamoto et al [10].

On the other hand, magnesium oxide (MgO) has been known as one of the metal oxide ceramics with high bactericidal effects, without the irradiation of light. The main mechanism of its antibacterial activity is proposed to be the generation of super-oxide anions (O_2^-) on the surface of MgO, and the increase in pH value with the hydration of MgO [11].

It seems that the doping of ZnO nanoparticles with Mg can affect antibacterial activity, as it might enhance ROS generation, change band gap energy, or the morphology of ZnO nanostructures. In our previous research, the effect of Mg doping on the physical and antibacterial properties of ZnO nanostructured thin films was studied [12]. In this work, we investigate dopant induced changes in the physical properties of ZnO:Mg nanoparticles. In addition, the antibacterial activities of nanosuspensions containing such nanoparticles are examined.

2. Experimental methods

ZnO:Mg nanoparticles were synthesized by refluxing a solution of $Zn(NO_3)_2 \cdot 6H_2O$ and magnesium chloride ($MgCl_2 \cdot 6H_2O$) in a mixture of absolute ethanol and deionized water (1:1). Ethylene glycol and acetic acid were used as polymerization and complexing agents, respectively. The homogeneous mixture was maintained under reflux at 100–110°C for 6 hours. After vaporizing the excess solvents, a white powder was obtained. Before characterization, the powders were first calcined at 450°C and then milled.

To make a nanosuspension for antibacterial tests, a certain amount of NPs was mixed with deionized water (1g/Lit), and stirred vigorously. In order to have homogenous nanosuspension, a ball-mill mixer was used. For sterilization, prepared nanosuspensions were autoclaved at 121°C and at a pressure of 20 barr for 20 minutes.

The typical XRD spectra of the prepared nanoparticles were characterized in the range of $20^\circ < 2\theta < 60^\circ$ with 0.04° step size. The morphology and size of the samples were examined by TEM. For investigating the chemical composition of the samples, Fourier Transport Infrared (FTIR), in the range of 400–4000 cm^{-1} , and EDS (Energy -Dispersive X-ray spectroscopy) analysis were carried out. For optical studies, the absorption spectra of the films were obtained by a UV-Vis spectrophotometer.

Antibacterial tests were performed by measuring the growth curve of bacteria in the presence of nanosuspensions [13]. In order to make sure about the antibacterial effectiveness of ZnO nanosuspension on different kinds of bacteria, the test was done on both gram positive and gram negative bacteria. The

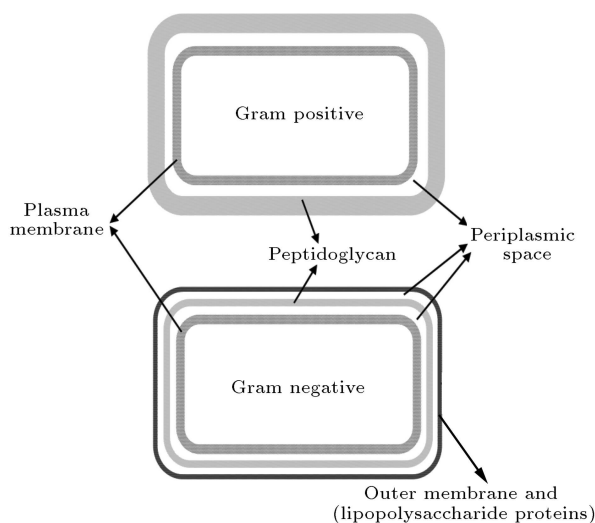


Figure 1. Cell wall structure of gram positive and gram negative bacteria.

difference between the gram positive (like *S. Mutans*) and gram negative (such as *E. coli*) bacteria is in their external structure. As seen in Figure 1, a gram positive bacterium has a thick layer of peptidoglycan, and a gram negative bacterium has a thin peptidoglycan layer and an outer membrane. Both the ROS generation and ion release can drastically alter and interfere with the function of this outer structure.

Therefore, we studied the antibacterial effect of ZnO nanosuspensions on *E. coli* (HB 101) as gram negative, and *S. Mutans* (PTCC1683) as gram positive, cultures. These two microorganisms were incubated in appropriate growth mediums; LB (Luria-Bertani) for *E. coli* and TSB (Trypton Soya Broth) for *S. Mutans*. The growth curves were obtained by measuring the time evaluation of the Optical Density (OD) of the samples. The measurements were performed at a 600 nm wavelength at the frequency of once an hour.

3. Results and discussion

3.1. Structural investigation

Figure 2 shows the XRD patterns of the samples. As can be clearly seen, ZnO particles possess a polycrystalline hexagonal wurtzite structure. No peaks originating from the MgO rock-salt structure were detected below an Mg concentration of 15%. This indicates that Mg^{2+} can be incorporated into the ZnO lattice with no phase segregation taking place, which was not unexpected, as the Mg atom has a similar ionic radii to that of the Zn atom (0.57 Å for Mg and 0.60 Å for Zn). The presence of the (002)-wurtzite peak, along with the (200)-cubic peak, at a dopant percentage of 15% suggests the coexistence of two phases. To gain further insight, the crystallite size, with the help of the

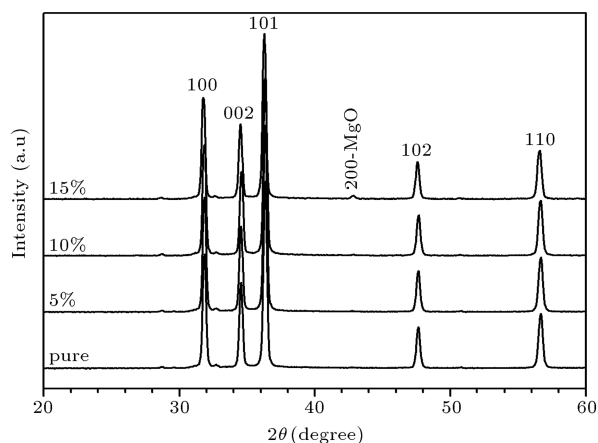


Figure 2. XRD patterns of ZnO:Mg nanoparticles with different Mg contents.

Sherrer relation, and the lattice parameter, c , of the samples, were calculated (Figure 3). The increase of Mg content results in a decrease in lattice constant. This could be due to structural adjustment, as the variation of lattice constant is related to the bond flex of the anion and cation, the radius difference of the substitutional ion, and the change in crystal structure [14].

Results of morphological examinations are depicted in Figure 4. Clearly, the introduction of Mg to ZnO nanoparticles leads to an increase in the particle size of the samples.

3.2. FTIR analysis

IR spectra of ZnO nanoparticles with different Mg concentrations are shown in Figure 5. The high intensity absorption peaks from the vibration of the hydroxyl group at $\sim 3400\text{ cm}^{-1}$ and $\sim 1600\text{ cm}^{-1}$ can be assigned to the O-H stretching vibrations, due to the absorbed water on the surface of the samples. The absorption in $\sim 2360\text{ cm}^{-1}$ is because of the presence of CO_2 molecules in the air [15].

For the pure sample, the intense absorption peak at $\sim 435\text{ cm}^{-1}$ is related to the stretching vibrations of

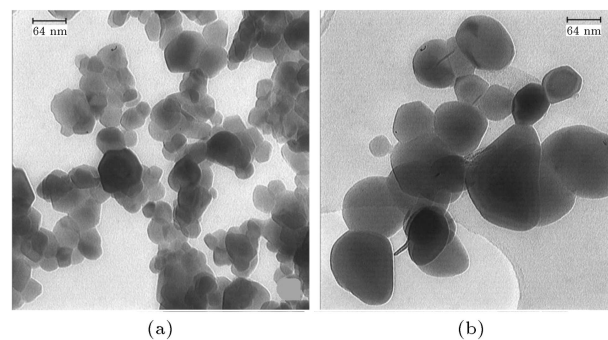
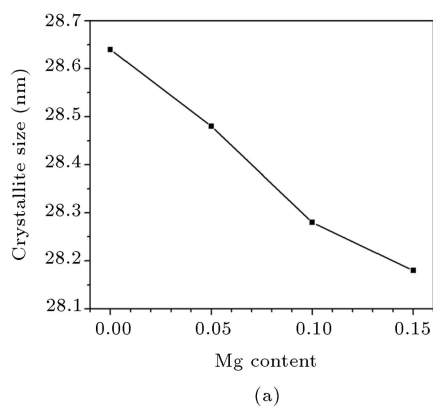


Figure 4. TEM image of ZnO:Mg nanoparticles: (a) Pure ZnO; and (b) ZnO:Mg with 15% Mg content.

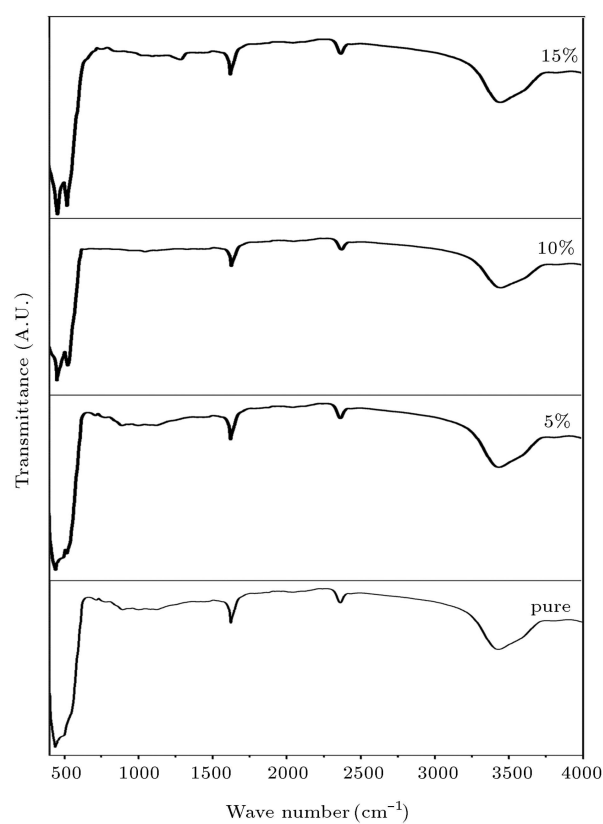


Figure 5. FTIR spectra of pure and doped ZnO samples.

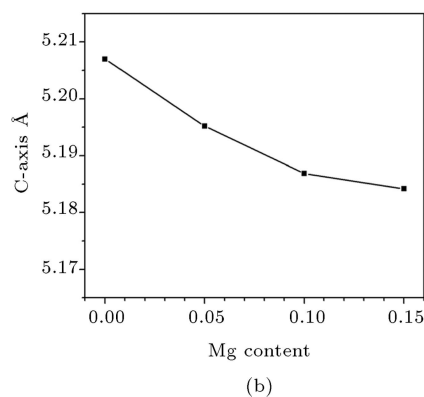


Figure 3. Structural changes in ZnO nanoparticles with the amount of Mg doping.

the Zn-O bond. With the incorporation of Mg dopant, an additional absorption peak at $\sim 520\text{--}535\text{ cm}^{-1}$ is observable, the intensity of which increases with the increase of dopant concentration. One can also observe a shift in the stretching bond position of Zn-O towards a higher wave number, from 435 to 465 cm^{-1} , with a corresponding increase in Mg molar concentration from

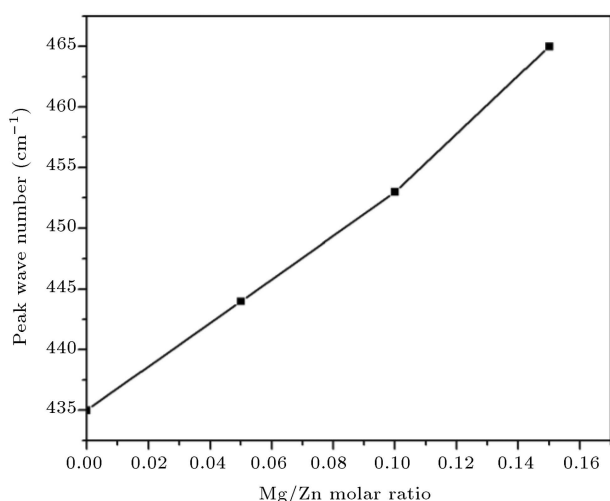


Figure 6. Shifting of stretching bond position of Zn-O with the increase of Mg content.

0 to 0.15. This shift is attributed to the substitution of Zn by the lighter Mg atoms [16]. Figure 6 shows the variation of the stretching bond position with Mg concentration.

3.3. EDS spectroscopy

For more investigation into the chemical composition of the samples, the EDS spectra of the pure and 15% Mg doped nanoparticles are depicted in Figure 7. Apart from the intense peak of Si and other peaks of Ca, Pd and Au, which are related to the chemicals in the substrate and conducting layer deposited on the samples, one can see Zn and O peaks in pure ZnO nanoparticles.

For the nanoparticles with 15% Mg content, a peak related to Mg is also observable.

3.4. Optical studies

In order to study the evaluation of band gap energy, the UV-Vis spectra of the samples were examined in absorption mode (Figure 8(a)). All samples showed a sharp absorption peak in the UV region. The observed shift in the absorption edge is from 372 to 339 nm , with the increase of dopant concentration from 0 to 15%, which clearly reflects the incorporation of Mg in the ZnO lattice. The results also show that the

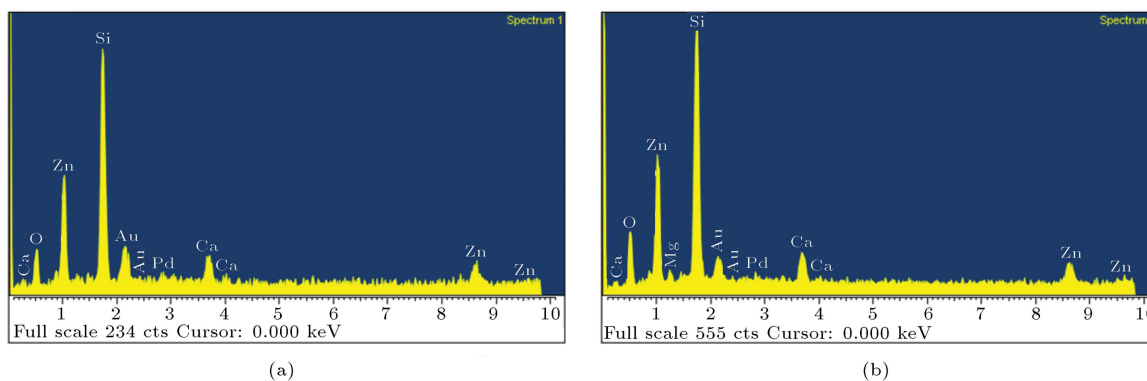


Figure 7. EDS spectra of the pure ZnO, and 15% Mg doped ZnO nanoparticles.

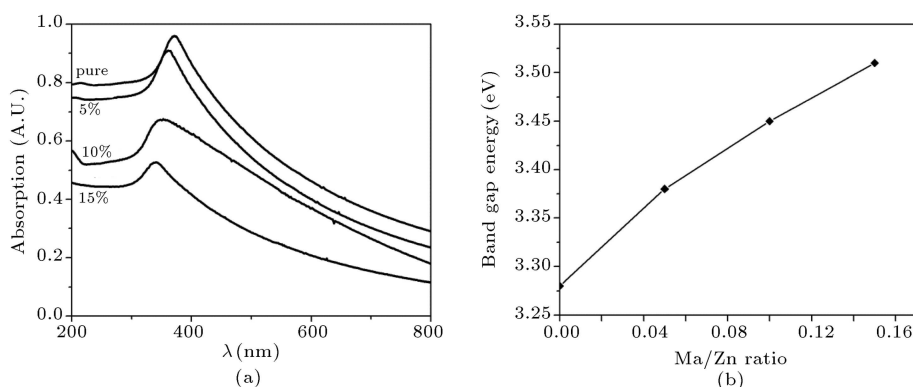


Figure 8. (a) Absorption spectra of nanoparticles of ZnO:Mg with different Mg contents. (b) Variation of band gap energy with increase of doping concentration.

increase in Mg concentration leads to an increase in transmittance. The direct band gap of the samples was calculated using the so called Tauc relation, as follows:

$$\alpha h\nu = A(h\nu - E_g)^n,$$

where α is the absorption coefficient, A is a constant, h is Planck's constant, ν is photon frequency, E_g is energy band gap and n is 1/2 for the direct band gap semiconductor. The results are shown in Figure 8 (b).

Band gap studies show that with the increase of Mg/Zn ratio, the optical band gap increase. This may be attributed to the fact that new defects are introduced after Mg atoms substitute Zn atoms and enter into the ZnO lattice, due to the electronegativity and ionic radius difference between Zn and Mg. As a result, the exciton bond increases with the increase in Mg content. Moreover, there are more electrons contributed by the Mg dopant, due to the lower electron affinity of Mg compared to Zn, which take up the energy levels located at the bottom of the conduction band. Since the Pauli principle prevents the states from being doubly occupied, and the optical transitions are vertical, therefore, the valence electrons require extra energy for excitation to higher energy states in the conduction band [17].

3.5. Antibacterial activity

Figure 9 shows the growth curve of *E. Coli* and *S. Mutans* in the presence of nanosuspensions of ZnO, gathering with negative control (pure bacterial culture without the addition of nanosuspension). As the value of the optical density (OD) at 600 nm represents the absorbance of the bacteria, an increase in the number of bacteria implies more light being absorbed by them. It can be seen that ZnO nanosuspensions considerably hinder the growth of bacteria. The antibacterial effect is more powerful for *E. coli* as a gram negative bacteria. This can be due to the difference in the membrane structure of gram positive and negative bacteria.

In the case of Mg incorporation, as seen in Figure 10, the presence of ZnO:Mg nanosuspension still

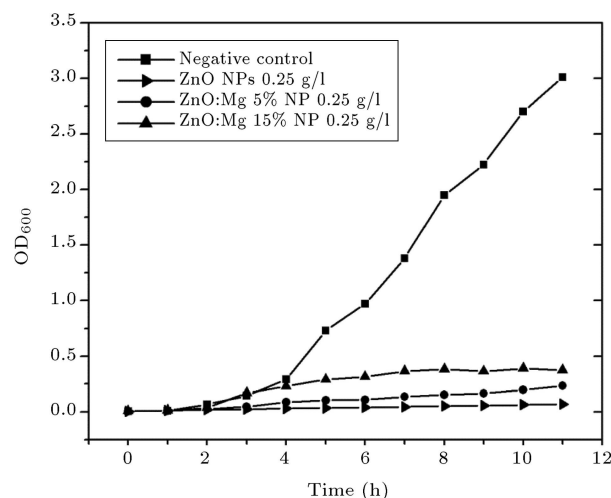
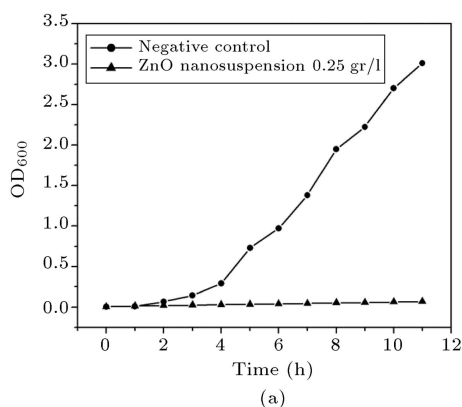


Figure 10. Growth curve of *E. Coli* in the presence of ZnO:Mg nanosuspension with different Mg contents.

hinders the growth of *E. Coli*. But, by addition of Mg, the antibacterial activity in ZnO nanosuspensions shows a delay of 5 hours. This could be attributed to an increase in particle size by doping, which reduces the surface-to-volume ratio. Moreover, the increase of band gap energy reduces the chance of photocatalytic activity, as one of the antibacterial mechanisms.

While, in our previous work, Mg doping enhances the antibacterial effectiveness of ZnO nanostructured thin films, in the case of nanosuspensions, the addition of Mg does not show an improving effect. This suggests that the antibacterial activity is highly sensitive to morphology and the method of fabrication of ZnO nanostructures.

4. Conclusion

ZnO:Mg nanoparticles were synthesized using a sol-gel method and the effect of doping on its structural, morphological and chemical composition and antibacterial activity was investigated. ZnO samples show a hexagonal Wurtzite structure, and no phase

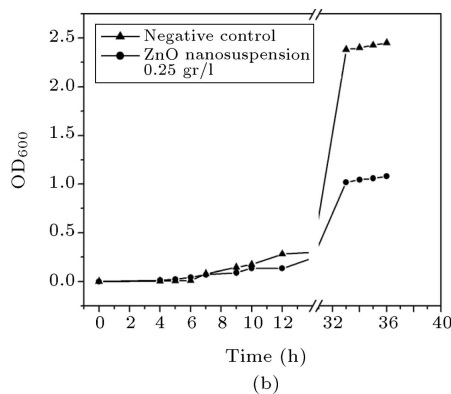


Figure 9. Growth curve of (a) *E. Coli*, and (b) *S. Mutans* in the presence of ZnO nanosuspension.

segregation takes place up to a 15% amount of doping. TEM images verify the formation of nanoparticles in the range of 30-55 nm. FTIR and EDS results also confirm the successful incorporation of Mg in the ZnO structure. From the optical studies, one can observe an increase in band gap energy by a corresponding increase in Mg content. The antibacterial activities of the nanosuspensions were tested against *Escherichia Coli* (gram negative) and *Streptococcus Mutans* (gram positive) cultures. ZnO:Mg nanosuspension shows high antibacterial activity, which slightly decreases with the amount of Mg.

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Biographies

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