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Special Issue

The Comparative Study of Antibacterial Activity of Zinc Oxide and Silver Doped Zinc Oxide

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Abstract: In this study, the comparative investigation of antibacterial activities of Zinc oxide and Silver doped Zinc oxide (Ag-ZnO) towards different strains of bacteria was made. ZnO was prepared by Co-precipitation method while Ag doping was done by green reduction method. For microcrystalline and surface morphological characteristics, samples were introduced to X-ray diffraction and scanning electron microscopy. Extensive growth of hexagonal wurtzite phase of ZnO with crystalline size of 38 nm was observed. On Ag doping crystalline size reduced to 24 nm. Energy dispersive spectroscopy confirms the doping of Ag in ZnO. Disc Diffusion Method was used to investigate antibacterial activity of ZnO and Ag doped ZnO by using Gentamycin as a standard. It was observed that silver doped ZnO showed better antibacterial activity.

Keywords: Silver doped ZnO, Antibacterial activity

1. Introduction: Zinc oxide is one of the most investigated metal oxides for different applications such as solar cell, photocatalytic activities, dye degradation, gas sensors, optoelectronic devices due to its unique properties like sensitivity, stability, high electron mobility and low cost [1-3].It's a wide band gap material (3.3 eV) having exciton binding energy of 60 meV[4]. Due to its non-toxic nature it has been widely used in many cosmetic products, face powders, hand creamsand lotions[5]. Considering the properties of ZnO, it has also been investigated for controlling pathogenic fungi and bacteria[6]**.** ZnO nanoparticles has been reported earlier as a potent antibacterial agent against various bacterial strain. The mechanism to kill the bacteria is mainly due to breakdown of cell wall and cell membrane[7,8]. The nanoparticles of ZnO ranging 30-40 nm in diameter can enter into the bacterial cell without damaging the cell wall and cell membrane but can induce the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which is causing the biochemical alteration of the bacterial cell and leading to death of bacterium[9.10]. Silver (Ag) has also been reported for antibacterial properties since ancient time and exploited for treating various ailments. The silver nanoparticles loaded with ZnO can show more antibacterial effects in comparison to individual effects of ZnO or Ag[10-12].

2. Material and Method:

Synthesis of ZnO and Ag loaded ZnO: Zinc Acetate Dihydrate was dissolved in double distilled water and kept under continuous stirring. After 30 minutes, drop wise solution of ammonia was added to maintain the pH at 9. On adding ammonia solution, white precipitation was observed. Solution was stirred for 5 hrs continuously at room temperature. Precipitate was filtered out and annealed at 600 °C. To synthesize Ag loaded ZnO nanoparticle, aqueous solution of silver nitrate was added to dispersed solution of ZnO nanoparticle in water. Leaf extract of *Pedalium murex* was added to solution. Leaf extract of *Pedalium murex* was prepared as reported earlier [13]. Solution turned to brownish yellow indicating the formation of Ag nanoparticle. Precipitate was centrifuged and annealed at 400 °C. As synthesized nanoparticles were subjected to XRD for phase and crystalline size analysis. UV-Visible spectroscopy was also done for optical characterization. Surface morphology was investigated by using FE-SEM. Elemental composition was studied by using EDS.

Antibacterial Studies: Four strains of bacteria - *Bacillus subtills* (121), *Escherichia coli* (40), *Salmonella typhimurium* (3231) and *Staphylococcus aureus* (7443) were procured from Institute of Microbial Technology, Chandigarh, Punjab for antibacterial analysis. All these strains were revived as per standard method followed by sub-culturing into Petri plates having nutrient agar medium (HiMedia). The antibiotic disc containing 10 mg of gentamycin antibiotic, 5mg/ml solution of ZnO nanoparticles and Ag loaded ZnO dipped discs for 30 minutes followed by air dry at room temperature in aseptic condition under laminar air flow were kept in Petri plates. Then these Petri plates were incubated for 24 hrs in incubator with 37^{0}C followed by measuring of zone of inhibition by caliper. Zone of inhibition was measured from center of the disc in triplicate set of experiments. Mean of zone of inhibition was measured followed by standard error mean [14,15]

3. Results and Discussion:

ZnO and Ag loaded ZnO was sussessfully synthesized by sol-gel method. Figure 1 shows the X-Ray diffraction pattern of sol-gel synthesized ZnO and silver loaded ZnO synthesized by sol-gel method. XRD peaks at 2θ, 31.7, 34.4, 36.2, 47.5, 56.6, 62.8, 66.3,67.9 and 69.06 ° correspond to Hexagonal wurtzite phase of ZnO (PDF 01-089-0511). No other phase of ZnO like Zinc Blend was observed. Additional XRD peaks at 38.1 and 44.3 ° cooresponding to planes (111) and (200) was also observed in silver loaded ZnO.

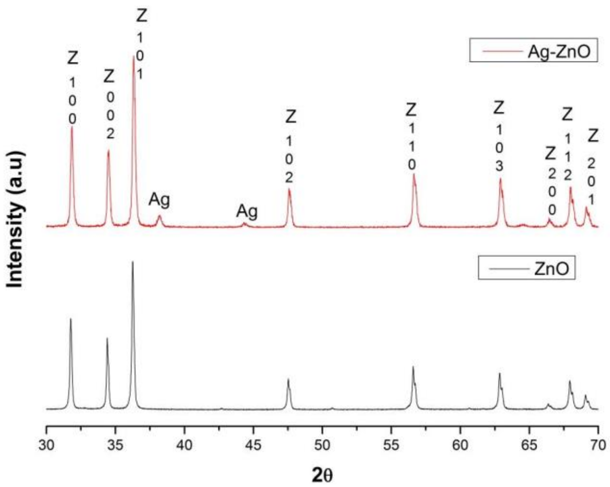

Figure 1: X-Ray diffraction pattern of ZnO and Ag loaded ZnO

Crystalline size was calculated by Scherrer's equation. The calculated average crystalline size of ZnO was 38 nm. In case of Ag loaded ZnO it was 24 nm so Ag loading did not cause any major effect on crystalline size of ZnO. The crystalline size of Ag nanoparticles was 24 nm which is quite lower than ZnO.

Figure 2 shows UV absorption of spectra of ZnO and Ag loaded ZnO. ZnO shows strong optical absorption in UV region with band gap 3.35 eV. In case of Ag loaded ZnO, addition absorption peak near aboout 440 nm corresponds to silver[13]. However, Ag loaded showed more absorption compared to ZnO.

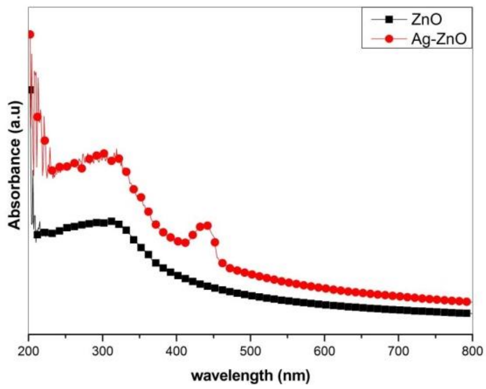

Figure 2: Absorption vs. wavelength curve of ZnO and Ag loaded Zno

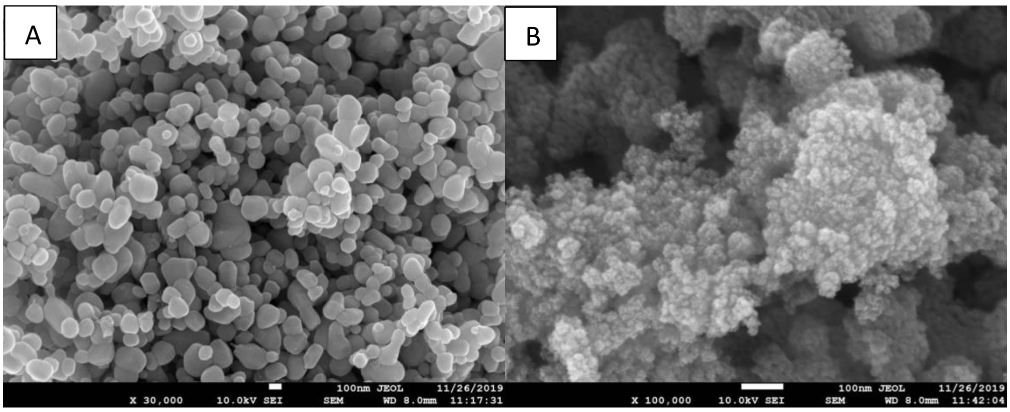

Figure 3:FE-SEM images of (A) ZnO and (B) Ag loaded ZnO nanoparticles

Figure 3 depicts the surface morphology of ZnO and Ag-ZnO loaded ZnO. In case of ZnO, particle size is bigger than crystalline size calculated by XRD. Clear grain boundaries can be seen. Morphology of particles is uniform throughout the sample and is free from any major structural defect. A little bit agglomeration of particles can be seen in case of Ag loaded ZnO. Also particle size is small and is compared to that of calculated by XRD. Particle size may be reduced due to extra stirring of samples and introduction of Ag particles. Loading of extra foreign particle may induce strain and can cause lattice strain leading to reduced size[16].

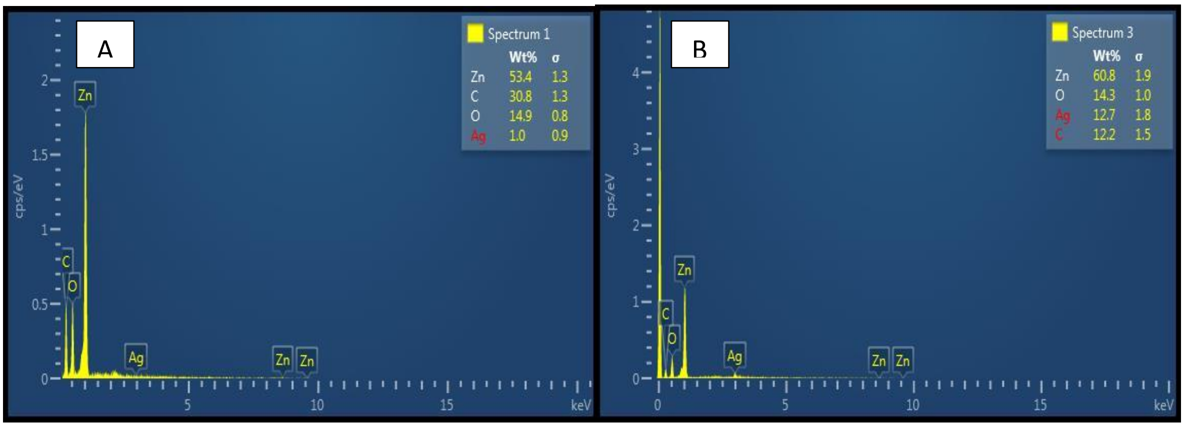

Figure 4:EDS pattern of ZnO (A) and Ag loaded ZnO (B).

Figure 4 depicts the EDS pattern of ZnO and Ag loaded ZnO. EDS pattern confirms the presence of Zn and O. Additional peak corresponding to carbon was due to Carbon tape used to record the spectra. Amount of silver was only 1% having error 0.9% and therefore, its presence may be ruled out. In case of Ag loaded ZnO, 12.2 wt % Ag was recorded. EDS pattern of Ag loaded ZnO confirms the loading Ag on ZnO.

In table 1 the antibacterial effects of ZnO and Ag loaded ZnO is shown.

Table: 1 Obtained Zone of inhibition in mm for four different bacterial strain mentioning Triplicate Mean ± Standard deviation

Zone of inhibition in mm			
Bacteria	Gentamycin	Only ZnO	Ag-ZnO
B. subtills(121)	19.67±0.57	7.46±0.41	12.06±0.41
E. coli (40)	17±1	8.13±0.50	13.63±0.55
S. typhimurium (3231)	13.67±1.52	5.8±0.35	9.06±0.41
S. aureus (7443)	18±1	6.46±1.22	13.5±0.7

The graphical representation of antibacterial effects of ZnO and Ag loaded ZnO compared with Gentamycin is shown in figure 5.

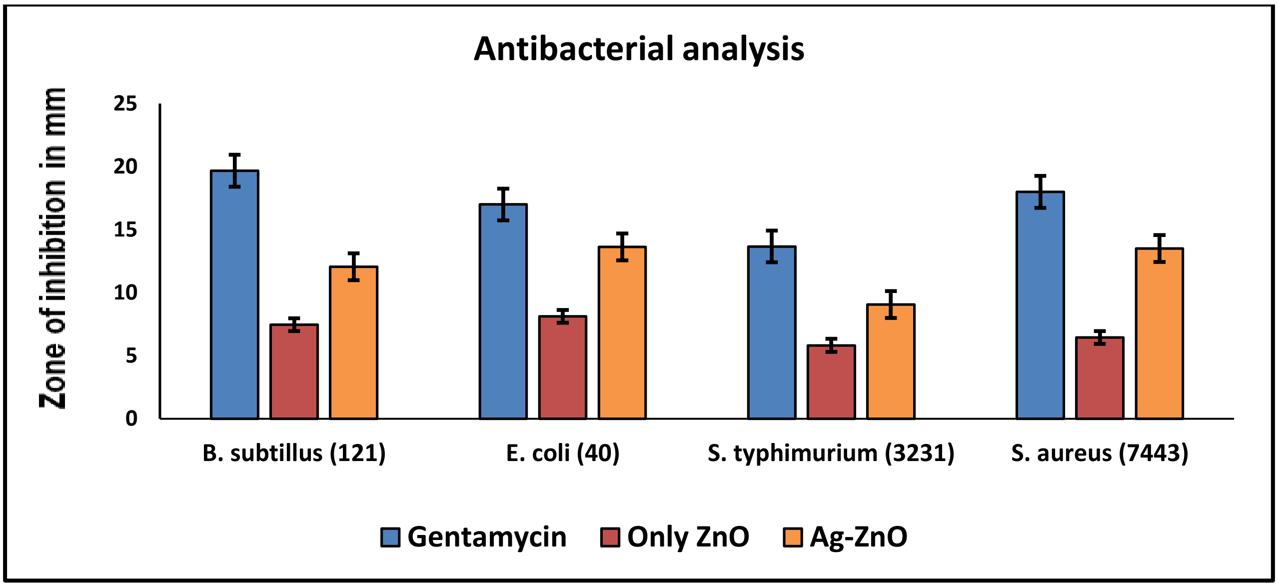

Figure 5: Antibacterial analysis of ZnO and Ag-ZnO compared with Gentamycin antibiotics

Obtained results shown that the Ag loaded Zno oxide nanoparticles has more antibacterial effects than the nanoparticles of ZnO alone. Similar antibacterial effects of ZnO nanoparticles have been reported by Reddy *et al.*in 2014 [7], Emami-Karvani*et al.* in 2011 [8] and Sirelkhatim*et al.* in 2015 [9]. Our research findings also suggest the antibacterial effects of ZnO nanoparticles and Ag loaded ZnO nanoparticles which signifies the application of ZnO nanoparticles in combination with antibiotics will show more potent antibacterial effects and can be explored as new antibiotics development.

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