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Enhancement and Evolution of Anti-Bacterial activity of ZnO NPs by doping with Silver

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ABSTRACT

In recent decades nanotechnology received a great importance because of its multidisciplinary role in various fields. Nano ZnO is one of most emerging nano metal oxide has been using in various applications such as fire retard, anti-microbe and anti-wrinkle etc. and showing good performance when doped with Silver. The Pure and Ag doped nano ZnO synthesized were subjected to characterization to know the morphology and physical properties. FTIR analysis was employed for the identification of functional group, X-Ray Diffraction is to identify the crystalline structure, SEM-EDX Analysis employed to know the morphological structure and size of the synthesized nanoparticles and elemental confirmation, and Transmission Electron Microscopy to determine the particle size and shape of synthesized nano particles. The synthesized Pure and Ag doped nano ZnO was screened for their anti-bacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* by disc diffusion method.

Key words: *Ag-ZnOnano particles, Wet-chemical method, FTIR, XRD, SEM-EDX, Enhancedanti-bacterial activity.*

Introduction

Nanotechnology has became one of the most fascinating and prevalent technology since a couple of decades and attracting a lot of interest in the area of energy production, nano electronics, nano medicine, biomaterials and consumer products. Nano metal oxides are playing a crucial role in lot of areas in material research, physics, chemistry, bio technology and medicine because of their structural characteristics and magnetic properties (King *et al.*, 2007), and they can provide a new strategy to combat antibiotic resistant micro-organisms (Logeswari *et al.*, 2015; Burda *et al.*, 2005; Thill *et al.*, 2006). Moreover, nano metal oxides are showing effective antibacterial activity and nontoxic compared to organic anti microbes because of their high surface area to volume ratio. Hence nano metal oxides have drawn the attraction of researchers to use in pharmaceutical and biomedical application as an alternative to microbial inhibitors (Hajipour *et al.*, 2012). In this connection researchers have shown interest on nano zinc oxide due to its extensive physical, chemical properties, which offer wide range of applications, such as energy storage, catalysis, molecular adsorption, ion- exchange, water treatment, chemical and biological sensing, photo catalysis, manufacturing of solar cells, sensors, electrical devices and optical coatings and imaging contrast agents (Satheesh *et al.,* 2017; Sibmah *et al.,* 2022; Nirmala *et al.,* 2011).

Present days the essentiality of antibiotic is a big challenge for public health because of spreading of infections severely. There are a variety of pathogenic microorganisms found in the clinical field, in which some common closely related species that cause a wide range of diseases and still a significant cause of death. Hence, new plan of actions needed to know and execute the next level of drugs to control micro-organisms, that must be effective and affordable therapeutic approaches (Satheesh *et al.,* 2019; Satheesh *et al.,* 2019; Anandan *et al.,* 2007; Salem *et al.,* 2005; Lewis *et al.,* 2005; Rosi *et al.,* 2005; Wu *et al.,* 2015).

In recent years hybrid nano particles have drawn a lot of attention in the field of nano technology due to changing the properties of individual particles along with providing new and enhanced functionality. However, still it is a difficulty to prepare smaller nano composites with high specific surface area to improve anti-bacterial activity. In this work we have reported the synthesis of pure and Ag doped nanoZnO, resulting in a large specific surface area. The investigation reveals that, after doping with Ag the nanoZnO particles were showing excellent antibacterial activity against gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* and gram-positive *Staphylococcus aureus*, *Bacillus subtilis* because of the synergistic effect of the silver and nano ZnO NPs (Zhong *et al.,* 2001; Alammar *et al.,* 2009; Aguire *et al.,* 2011; Vidya *et al.,* 2015; Yang *et al.,* 2014)

Experimental

Materials and Methods

All the chemicals utilized in this work are Analytical-grade and utilized directly without any additional purification. Zinc nitrate, silver nitrate, and sodium hydroxide were acquired from Sigma Aldrich, and doubly distilled water was utilized whenever required throughout the experiment. All the reactions were conducted in an ambient condition at room temperature.

Procedure for the Synthesis of nano Zinc oxide

0.2 M solution of zinc nitrate hexahydrate ($Zn(NO_3)_2$"$6H_2O$) was taken in 500 ml of beaker and added 0.4M Sodium hydroxide (NaOH) drop-wise by stirring continuously at room temperature to form metal hydroxides. The stirring procedure was continued at 85 ^oC for 6 hours to obtain as-synthesized powder. Thus, the as-synthesized powder was calcined in Muffle furnace at 600 ^oC for 2 hours and furnace cooled. The resulting product was characterized by XRD, SEM and EDAX (Satheesh *et al.,* 2019).

Procedure for the Synthesis of Ag doped nano Zinc oxide

0.3 M solution of zinc nitrate hexa hydrate ($Zn(NO_3)_2.6H_2O$) was taken in 500 ml of beaker, added 0.025g of silver nitrate ($AgNO_3$) with continuous stirring for 30 min. and 0.4M Sodium hydroxide (NaOH) was added drop-wise by stirring continuously at room temperature to form metal hydroxides. The stirring procedure was continued at 85^oC for 6 hours to obtain as-synthesized powder. Thus, the as-synthesized powder was calcined in Muffle furnace at 600^oC for 2 hours and furnace cooled. The resulting product was characterized by XRD, SEM and EDAX.

Characterization

FTIR (Fourier Transmission Infrared) spectroscopy (Perkin Elmer- Spectrum RX-IFTIR) was employed to identify the functional groups present in the synthesized product. The X-Ray diffraction patterns were recorded on Bruker X-Ray Diffractometer using graphite filtered CuK radiation (λ = 1.54 A^o) at 40 KV with scanning rate of 3/min (from 2θ = 20-80°). Morphology and Size of the particles were determined by the 200 KeV Transmission Electron Microscope (TECNAI 200 Kv TEM- Fei, Electron Optics). Elemental composition of particles was determined by using SEM-EDAX (JEOL JSM 5600, EDS Model: INCA Oxford).

Fourier Transform-Infra Red (FT-IR) Spectral Analysis of Pure and Ag-ZnO

Figure 1shows the FTIR spectra of pure and silver doped zinc oxide, the figure showing that the FTIR spectrum for Ag doped ZnO is similar to that ofpure zinc oxide. FT-IR analysis was done using optimized parameters to determine the bond structure and related functional groups of the synthesized ZnOnano particles. The FTIR absorption spectra of the synthesized particles were identified in the 4000-400 cm^{-1} wave number range (Figure 1). The band located at 450 cm^{-1} attributed to the Zn–O stretching mode of the ZnO lattice. In these limits no additional peaks appears due to the doping silver, probably it arises from homogeneous dispersion of Ag particles on the surface of zinc oxide without any cluster formation. The band that observed near 3400 cm^{-1} was assigned to vibration mode of O-H group. A weak band observed near 2900 cm^{-1}was assigned to C-H modes. The Sharp band that observed near 1400-1600 cm^{-1}were assigned to stretching vibration modes for C=O groups (Zhang *et al.,* 2011; Iqbal *et al.,* 2021; Irshad *et al.,* 2019; Ishaq *et al.,* 2018; Deepika *et al.,* 2020.)

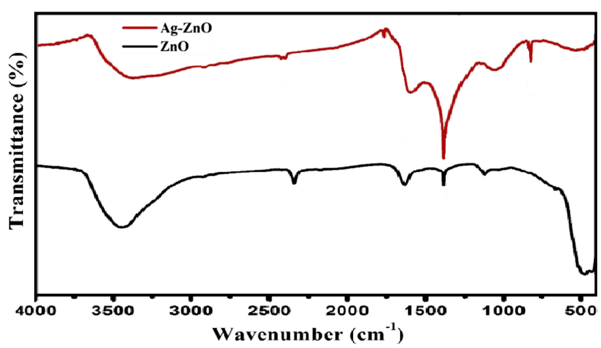

Fig. 1. FT-IR spectrum of Pure and Ag doped nanoZnO

X-Ray Diffraction (XRD) Analysis of Pure and Ag-ZnO

Figure 2 shows the XRD spectrum of pure and Ag doped Zinc oxide nano particles. The diffraction pattern of Ag-ZnOnano particles were almost similar to that of pure ZnO, which determines about crystallinity and good size of the particles. The diffraction angle of 2θ scanned in the range of 20-80°. The peaks showed at 31.80°, 34.47°, 36.29°, 47.58°, 56.62°, 62.90°, 66.40°, 67.98°, 69.11°, and 76.99°, determines the reflecting planes at (100), (002), (101), (102), (200), (112), (103), (110), (201), and (202) respectively. All the diffraction patterns shows the strong resemblance with the reported JCPDS (Joint Committee on Powder Diffraction Standards) belonging to hexagonal wurtzite crystal phase of synthesized particles.

Scanning Electron MicroscopyAnalysis of Pure and Ag-ZnO

Figure 3(a, b) and 3(c, d) shows the SEM images of

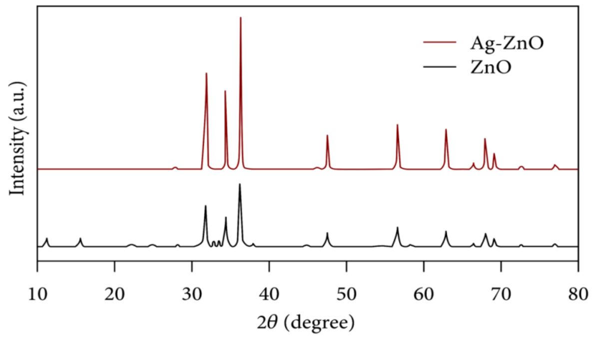

Fig. 2. XRD pattern of Pure and Ag doped nanoZnO

the synthesized Pure and Ag-ZnO nano particles respectively. From SEM images it is clear that the morphology of ZnO NPs has changed after doping with Ag. The SEM images determines that the synthesized Ag-ZnO nano particles were well agglomerated with different sizes present in hexagonal shape. However, in higher magnification, it shows the cubic structure, that indicates the presence of Ag NPs and also is highly porous, that represents that Ag NPs were coated on ZnO surface (Snehal *et al.,* 2022; Nadia *et al.,* 2022; Chauhan *et al.,* 2012; Susan *et al.,* 2014).

Transmission Electron Microscope (TEM) Analysis of Pure and Ag-ZnO

The samples of synthesized nano particles were systematically analyzed by TEM to notify the morphology and actual size of the particles. The TEM image of nanoZnO (Figure 4 (a,b)) shows the presence of *hexagonal* wurtzite accumulated with a dimension of

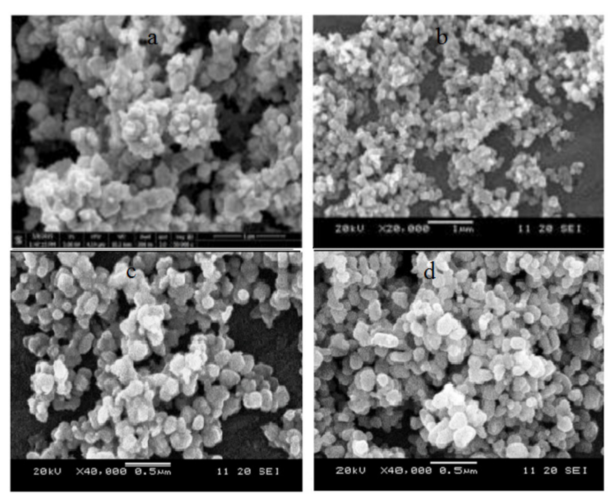

Fig. 3(a,b,c,d). SEM images of Pure and Ag doped nanoZnO

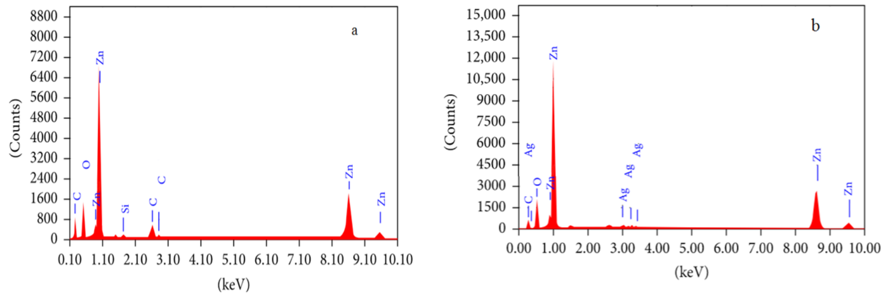

Fig. 4(a,b). EDX spectrum of Pure and Ag doped nanoZnO

~50 nm. The existing size of the nanoparticles was found to be around 30 nm. Figure 4(c, d) show the TEM images of Ag-ZnO nano particles, which shows the sizes of the Ag-ZnOnano particles were in the range of 30–110 nm. The existing size of the nanoparticles was found to be around 70 nm.

Energy Dispersive X-Ray(EDX) Analysis of Pure and Ag-ZnO

The elemental composition of the synthesized nanoparticles were confirmed by EDX analysis. Figure 4a and 4b showing the EDX images of pure and Ag doped ZnOnano particles, which determines the presence of Zn, Ag and O and there is no other impurities were detected (Geetha *et al.*, 2016).

Biological Activity

The antibiotic potency can be determined using the microbial assays. The basic principle of microbial assay lies in comparison of the inhibition of growth of bacteria by measuring concentration of the product to be investigated with that produced by known concentration of the antibiotic having a known activity. The methods used for assay are cup plate method and disc diffusion method. The cup plate method is based on the diffusion of an antibiotic from a cavity through the solidified agar layer of a petridish. Growth of inoculated microbe is inhibited entirely in a circular zone around a cavity containing a solution of the antibiotics.

Preparation of Pure and Ag –ZnOnano particles for the evolution of anti-bacterial activity

Initially the synthesized Pure and Ag-ZnOnano particles were sterilized at 150 °C for 3 h separately. 0.5g of each was taken, 0.1g of acacia gum, 3 ml of ultrapure water were added and mixed well in a mortar and pestle. The resulting suspension was subjected to sonication for 30 minutes, which was diluted later and utilized for anti-bacterial evaluation.

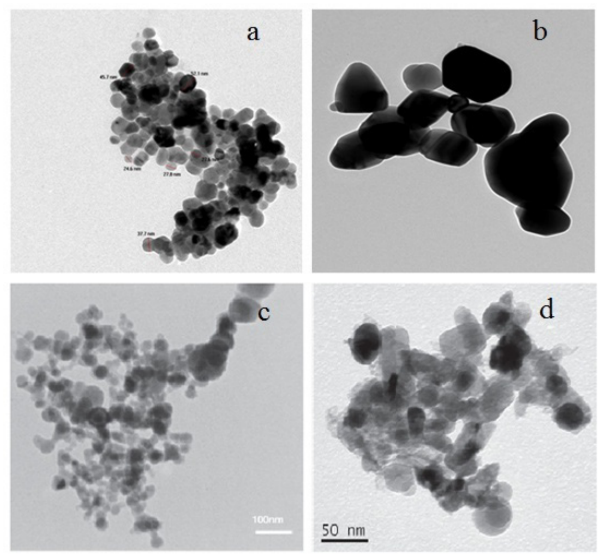

Fig. 5 (a,b,c,d). TEM images of Pure and Ag doped nanoZnO

Table 1. Zone of inhibition of pure and Ag doped nanoZnO against Micro-organisms

S. No.	Name of Micro organism	MTCC No.	Concentration of the nanoZnOsample			Concentration of the nano Ag-ZnOsample		
			10µl	20µl	30µl	10µl	20µl	30µl
			Zone of inhibition (mm)			Zone of inhibition (mm)		
1	*Escherichia coli* (Gram –ve)	2692	√ 2.0	√ 2.3	√ 2.5	√ 2.3	√ 2.6	√ 3.2
2	*Pseudomonas aeruginosa* (Gram –ve)	2453	√ 0.8	√ 1.2	√ 1.4	√ 1.2	√ 1.5	√ 2.2
3	*Staphylococcus aureus*(Gram +ve)	902	√ 0.4	√ 0.8	√ 1.2	√ 0.8	√ 1.2	√ 1.6
4	*Bacillus subtilis* (Gram +ve)	441	√ 1.6	√ 2.4	√ 2.6	√ 1.8	√ 2.6	√ 3.0

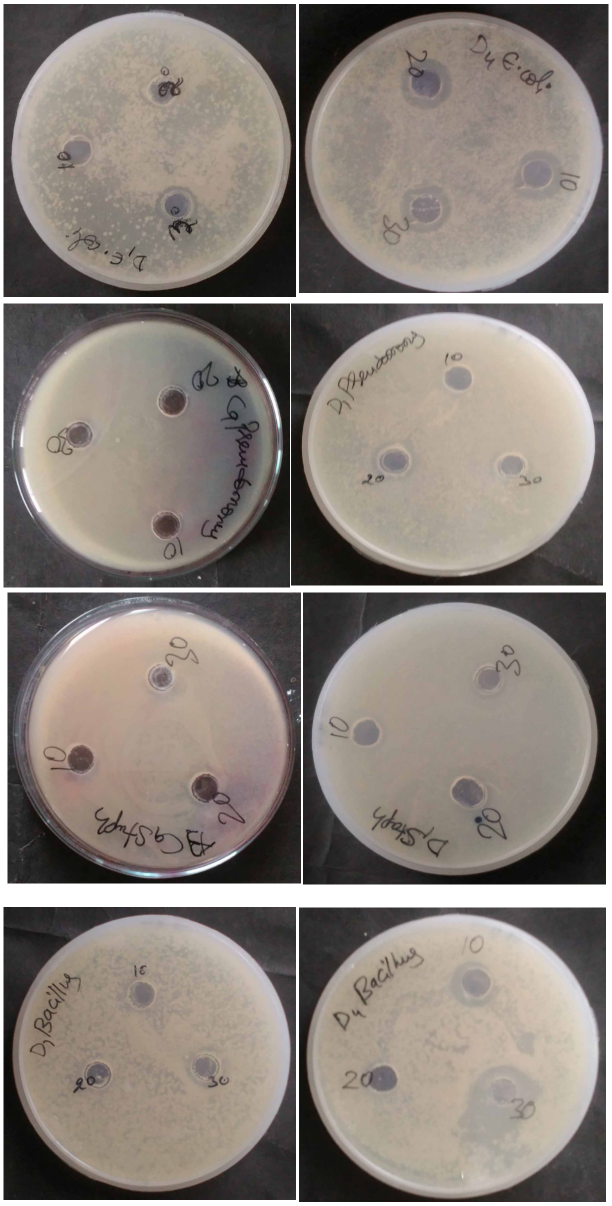

Fig. 6. Representative images of Anti-Microbial activity of Pure and Ag-dopednanoZnO

Antibacterial activity of Pure and Ag doped nanoZnO against Gram positive and Gram negative bacteria is shown in Figure 5. The evolution results reveals that the anti-bacterial activity of nanoZnO particles were enhanced after doping with Ag, and also the zone of inhibition increased as the concentration of the NPs increased.

Conclusion

Pure and Ag doped nano zinc oxide particles were synthesized by wet chemical process and subjected to characterization, the results confirms the size of ZnO particle were under the nano range (~50 nm) and the size of Ag doped ZnOnano particles were under thenano range (~70nm) having hexagonal wurtzite crystal structure. The antibacterial activity of the synthesized pure and Ag doped nano ZnO was assessed using the different concentration of the sample i.e., low, intermediate, high. The MIC (Minimum Inhibitory Concentration) of these sample that can inhibit bacterial growth is 10 µl, 20 µl and 30 µl respectively. Thus the above sample is able to show antibacterial activity on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*. Based on the investigation results it was clear that the anti-bacterial activity of ZnO NPs has enhanced after doping and also the zone of increased with increasing the concentration of NPs. Therefore, it may be concluded that the bio-functionalized hybrid metal oxide nano particles have a higher bio-cidal effectiveness in resisting bacterial growth, thus our findings reveal that Synthesis and Characterization possess a great role in the antimicrobial activity increment of the synthesized Metal oxide nanoparticles while it was surface functionalized with them. This may lead to propose valuable inventions in the field of antimicrobial systems as well as other medical applications in the future.

Conflict of Interest: None.

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