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Antibacterial Evolution of ZnO Nanoparticles Synthesized via Wet-Chemical Method

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Abstract

In present days the essentiality of antibiotics is a big challenge for public health because of the spreading of infections severely. Nano metal oxides show effective antibacterial activity and are non-toxic compared to organic anti-microbes because of their high surface area to volume ratio. Nano ZnO is one of the most emerging nano metal oxides that has been used in various applications such as fire retard, anti-microbe, anti-wrinkle, etc. Nano zinc oxide was synthesized by the wet chemical process using zinc nitrate hexahydrate as a precursor. The synthesized nano ZnO powder was 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 is employed to determine the morphological structure and size of the synthesized nanoparticles and elemental confirmation, and TEM is used to determine the particle size and shape of synthesized nanoparticles. The synthesized nano ZnO is screened for its antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis* by disc diffusion method.

Keywords: Nano ZnO; Wet-chemical method; Antibacterial activity.

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1. Introduction

In recent decades nanotechnology received great importance because of its multidisciplinary role in the rapidly growing fields of bio-nanotechnology and nanomedicine and it can provide a new strategy to combat antibiotic-resistant microorganisms [1]. A number of studies have suggested that nanoparticles (NPs) can be used as therapeutic agents against microbes due to their distinctive physical, chemical, magnetic , and mechanical properties. These favorable properties can be assigned to their enumerated size, shape, and efficient biological properties. As a result, NPs have drawn a lot of attraction in the development of novel bactericidal agents [2,3]. Generally, the size of the bacterial cell is in the micrometer range, whereas the pores in its outer cellular membranes are in the nanometer range. Since the materials at the nanoscale can penetrate into bacterial cells and damage them by producing toxic oxygen that results in a successful inhibition of the growth of bacteria [4-7]. Nanometal oxides play a crucial role in a lot of areas in material research, physics, and chemistry because of their structural characteristics and magnetic properties [8-12]. Moreover, nanometal oxides show effective antibacterial activity and nontoxic compared to organic anti-microbes because of their high surface area to volume ratio. Hence nano metal oxides drawn the attraction of researchers for use in pharmaceutical and biomedical applications as an alternative to microbial inhibitors [13-16].

Nowadays the essentiality of antibiotics is a big challenge for public health because of the spreading of infections severely. There are a variety of pathogenic microorganisms are 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 plans of actions are needed to know and execute the next level of drugs to control microorganisms, which must be effective and affordable therapeutic approaches [17-20]. In this connection researchers have shown interest in nano ZnO due to its extensive physical, and chemical properties, which offer a 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 [21-25]. In this article we have reported the synthesis of nano ZnO by a wet chemical process and subjected it to characterization to know the morphology and physical properties by Fourier Transform Infrared (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX), and Transmission Electron Microscopy (TEM) analysis. The synthesized nano ZnO is screened for their antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis* by disc diffusion method.

2. Experimental

2.1. *Materials and methods*

All the chemicals utilized in this work are of analytical-grade and utilized directly without any additional purification. $Zn(NO_3)_2$ and NaOH are acquired from Sigma Aldrich, USA, and throughout the experiment doubly distilled water is utilized when ever required. ZnO nano particles are synthesized by wet chemical method using Zinc nitrate hexahydrate as precursor. The reason for choosing wet chemical method for the synthesis of nano ZnO is to achieve nano particles of appropriate size of NPs.

2.2. *Synthesis of nano ZnO*

All the reactions were conducted in an ambient condition at room temperature. 0.2 M solution of $Zn(NO_3)_2$ and 0.4 M NaOH were employed to synthesize nano ZnO particles by wet chemical process. In a 500 mL of beaker $Zn(NO_3)_2$ was dissolved in water and added sodium hydroxide drop-wise at room temperature by stirring continuously to form metal hydroxides. The stirring procedure was continued at 85 °C for 6 h to obtain as-synthesized powder (Fig. 1). Thus, the as-synthesized powder was calcined in Muffle furnace at 600 °C for 2 h and furnace cooled. The resulting product was characterized by XRD, SEM and EDAX [20-22].

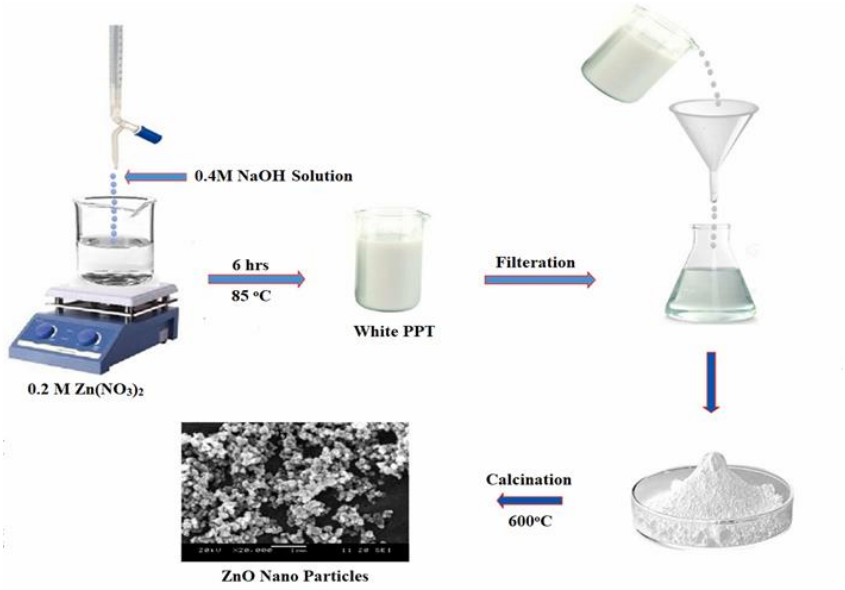

Fig. 1. Synthesis diagram of ZnO NPs.

2.3. *Characterization of catalyst*

FTIR (Fourier Transmission Infrared) spectroscopy (Perkin Elmer- Spectrum RXI-FTIR, USA) 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 Á) at 40 KV with scanning rate of 3/min (from 2θ = 20-80°). Optical absorption spectra were recorded on a UV-Vis spectrometer (Model, Shimadzu, Japan). Morphology and Size of the particles were determined by the 200 KeV Transmission Electron Microscope (TECNAI 200 Kv TEM- Fei, Electron Optics, China). Elemental composition of particles was determined by using SEM-EDAX (JEOL JSM 5600, EDS Model: INCA Oxford, UK).

3. Results and Discussion

3.1. *FT-IR*

FT-IR analysis was done using optimized parameters to determine the bond structure and related functional groups of the synthesized ZnO NPs. The FTIR absorption spectra of the synthesized particles were identified in the 4000-400 cm^{-1} wave number range (Fig. 2). The band located at 510 cm^{-1} attributed to the Zn–O stretching mode of the ZnO lattice [23-28]. The bands at 3276 cm^{-1} attributed to the O-H mode of vibration. C=O exhibits strong asymmetric mode of vibration at 1635 cm^{-1}.

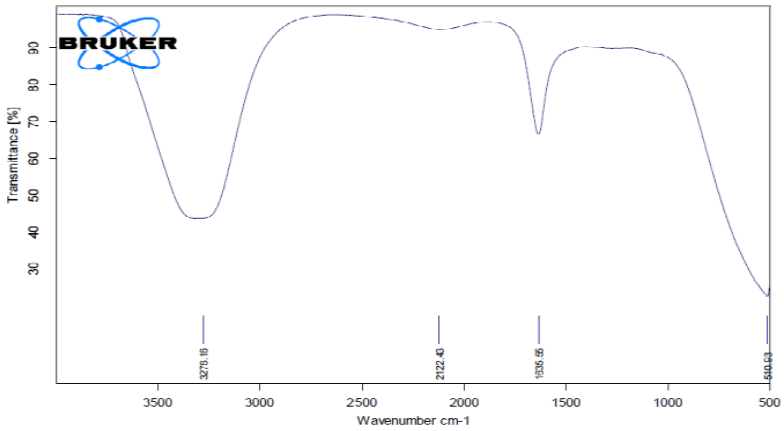

Fig. 2. FT-IR spectrum of nano ZnO.

3.2. *X-Ray diffraction (XRD)*

Fig. 3 shows the XRD spectrum of nano ZnO. The sharp diffraction peak determines about crystallinity and good size of the particles. The diffraction angle of 2θ scanned in the range of 20-80° shows peaks at 31.71, 34.37, 36.19, 47.49 and 56.5, determine the reflecting planes of (100), (002), (101), (110), and (200) respectively. All the diffraction peaks shows the strong resemblance with the reported JCPDS (Joint Committee on Powder Diffraction Standards) belonging to hexagonal wurtzite crystal phase of ZnO.

3.3. *SEM-EDX*

Figs. 4 and 5 shows the SEM and EDX of the synthesized nano ZnO respectively. The morphological structure of the synthesized ZnO nano particles was confirmed by SEM and the elemental composition of the synthesized nanoparticles was confirmed by EDX [29-32]. The SEM image shows that NPs with size less than 100 nm were formed. Individual particles with clear boundary indicates high collodal stability even in the dried state. The EDX spectra determines the presence of Zn and O and there is no other impurities were detected (Fig. 5).

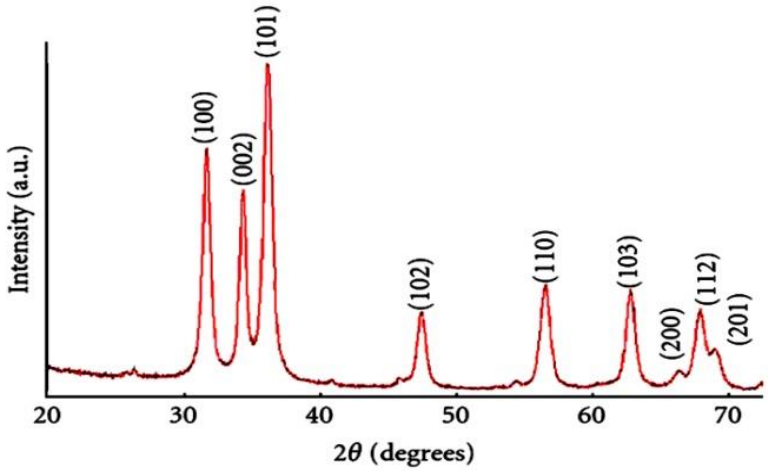

Fig. 3: XRD pattern of nano ZnO.

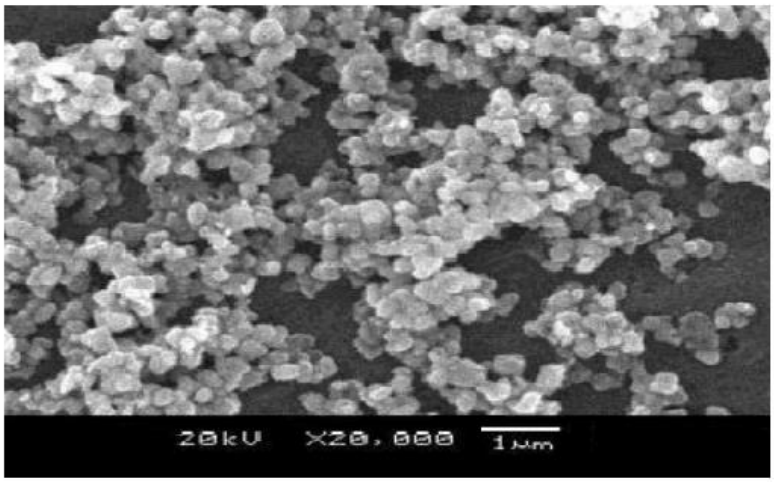

Fig. 4. SEM images of nano ZnO.

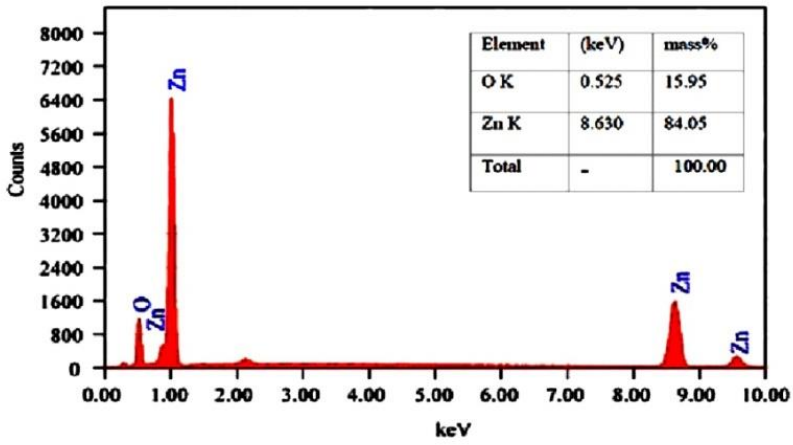

Fig. 5. EDX spectrum of nano ZnO.

3.4. *TEM*

Fig. 6 shows the TEM images of nano ZnO. The samples were systematically analyzed by TEM to notify the morphology and actual size of the particles. The TEM image of nano ZnO shows the presence of hexagonal wurtzite [33,34] accumulated with a dimension of ~50 nm. The existing size of the NPs was found to be around 30 nm.

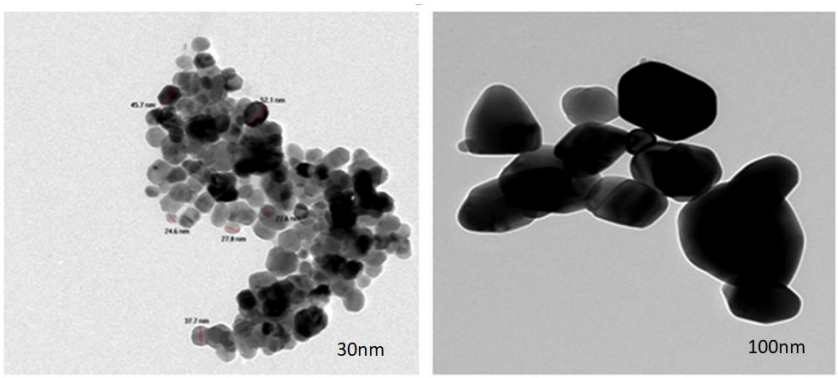

Fig. 6. TEM images of nano ZnO.

3.5. *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 [10,17,31]. Materials used for antimicrobial activity of ZnO nanoparticles are nutrient broth 1.3g, nutrient agar 5.6 g, Agar-agar 0.5 g, petriplates, antibiotic discs, cotton swabs, zinc oxide NPs sample, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*. Antimicrobial activity of nano ZnO obtained by wet chemical method against Gram positive and Gram negative bacteria is shown in Fig. 7. The results show the excellent antibacterial activity of the samples which was found to be improved with increase in concentration of ZnO NPs. The antibacterial activity of nano ZnO against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* maximum whereas that against *Staphylococcus aureus* was minimum as shown in Fig. 7. The investigation reveals that the zone of inhibition increased as the concentration of the sample compound increased.

Table 1. Zone of inhibition of nano ZnO against microorganisms.

S. No.	Microoranism	MTCC No.	Sample (µL)		
			10	20	30
			Zone of inhibition (mm)		
1	*Escherichia coli* (Gram –ve)	2692	V 2.0	V 2.5	V 2.7
2	*Pseudomonas aeruginosa* (Gram –ve)	2453	V 0.8	V 1.2	V 1.4
3	*Staphylococcus aureus* (Gram +ve)	902	V 0.4	V 0.8	V 1.2
4	*Bacillus subtilis* (Gram +ve)	441	V 1.6	V 2.4	V 3.2

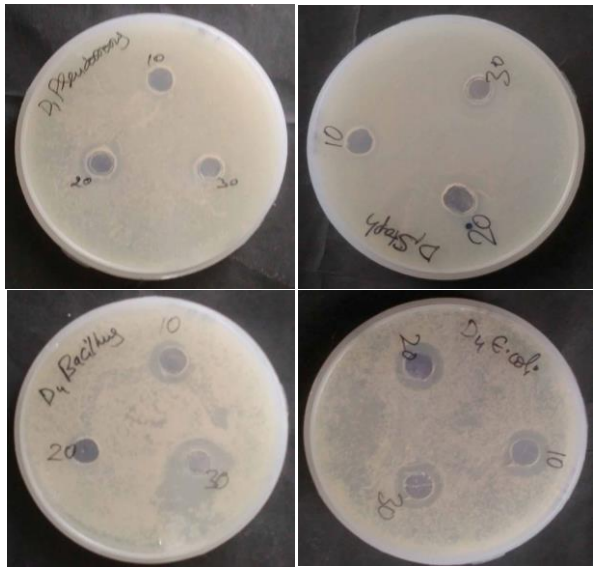

Fig. 7. Representative figures of anti microbial activity of nano ZnO.

4. Conclusion

Nano ZnO particles were synthesized by wet chemical process and the characterization results confirms the size of ZnO particle is under the nano range (~50 nm) having spherical shape and hexagonal wurtzite crystal structure. The antibacterial activity of the of the synthesized nano ZnO was assessed using the different concentration of the sample i.e., low, intermediate, high. The MIC 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*. Therefore, it may be concluded that the bio-functionalized metal oxide NPs have a higher bio-cidal effectiveness in resisting bacterial growth, thus our findings reveals 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.

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