Volume : 4 | Issue : 6 | June 2015 • ISSN No 2277 - 8179

Research Paper

Synthesis, Characterization and Determining the Antibacterial Effect of Zno Nanopowder Against the Pathogenic Bacteria's

Physics

KEYWORDS : *ZnO, Co-precipitate, Pathogens, Antibacterial activity, photoluminescence.*

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ABSTRACT

In this paper, ZnO nanopowder was prepared by co-precipitation method. The sample was characterized by X-ray diffraction (XRD), Scanning Electron Microscope (SEM), UV-Vis spectroscopy, Fourier transform infrared (FTIR) spectroscopy and photoluminescence. The average grain size of the prepared ZnO was determined by XRD. The purity of ZnO nano material crystalline sample was confirmed by photoluminescence. The antibacterial efficiency of ZnO nanopowder was studied using disc diffusion method. ZnO Nano powder has showed highest impact on the common animal pathogen E.coli and K. pneumonia.

1. Introduction

ZnO is a direct wide band gap semiconductor (Eg = 3.4 eV). The high exciton binding energy (60 meV) of this material ensures that it is a promising candidate for stable room temperature luminescent and lasing devices. Moreover, ZnO is a bactericide and inhibits both Gram positive and Gram negative bacteria [1]. The nanopowder possesses unique physico-chemical, optical and biological properties which can be manipulated suitably for desired application [2]. Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level [3]. Zinc oxide has significant attention due to its various applications such as UV light-emitting diodes, laser diodes and catalysts [4]. The high exciton binding energy of ZnO would allow for excitonic transitions even at room temperature, which could mean high radiative recombination efficiency for spontaneous emission as well as a lower threshold voltage for laser emission [5]. ZnO is widely used to treat a variety of skin conditions, in products such as baby powder, barrier creams to treat diaper rashes and in calamine cream, antidandruff shampoos and antiseptic ointments [6]. The ZnO nano structures are being widely investigated because of their great potential in electronic [7], photonic [8], spintronics [9, 10], biosensor [11, 12], drug delivery [13, 14], bio- imaging [15,16] and biomedical [17] applications

In the present study, we report the synthesis of ZnO nanopowder using co-precipitate method. The characterization of ZnO nanoparticles using X-ray diffraction, Fourier transform infrared (FTIR), UV-Vis absorbance and photoluminescence spectra is discussed and also investigate the effect of zinc oxide nanoparticles on the antibacterial activity of both cattle and poultry pathogenic bacteria stains for 24 and 48 hours.

2. Materials and methods

2.1. Synthesis of ZnO powder

An amount of 6.6 gm of Zinc acetate dehydrate ($(Zn(CH_3COO)_2.2H_2O$) was dissolved in 50 ml of distilled water with continuous stirring until the formation of a homogeneous solution. It was used as precursor solution, to which 4.6gm of Sodium Hydroxide (NaOH) dissolved in 50ml of distilled water was added slowly and the resulting white precipitate obtained, was stirred vigorously at ambient temperature for a duration of 1 hour. The precipitate was washed several times with deionised water and with ethanol to remove the impurities. Finally, the product was calcinated at 500 °C for 4h to get it in the powder form.

The X-ray Diffraction (XRD) spectra were recorded using Bruker D8 advanced X-ray Diffractometer with Cukα_1 (1.54060A°) as a source. The SEM micrographs of the experimental samples have been obtained using Scanning Electron Microscope (INPECT-F Model). The absorbance of ZnO was found out at room temperature using Spectrophotometer (Shimadzu UV-Vis 1800). Besides, the Photoluminescence spectra of ZnO nanoparticles were also recorded by Spectrofluorometer (Perkin Elmer LS55)

2.2. Antibacterial Assay

Antibacterial assay of ZnO nanopowder was carried out using the method suggested by Bauer *et. al.*,[18]. There are about 6 different [Escherichia coli (Mastitis causing species), Klebsiella pneumonia (Mastitis causing species), Pseudomonas aeroginosa (Mastitis causing species), Staphylococcus aureus (Mastitis causing species), Streptococcus agalactiae (Mastitis causing species), and Salmonella typhimurium (Enteritis and Cystitis causing species)] bacterial pathogens (cattle and poultry) were obtained from Veterinary College and Research Institute, Namakkal, Tamil Nadu and used in the present study.

2.3. Disc Diffusion Method

Preparation of sterile disc

Whatman's No.3 filter paper was punched into 5 mm (diameter) disc form and they were sterilized, each sterile disc was incorporated individually with ZnO nanopowder solution using micropipette. Precautions were taken to prevent the flow of the solution from the discs to the outer surface and the discs were allowed to dry in air. After sometimes another doses of extracts were applied on discs and they were stored at 4°C.

Assay of antimicrobial activity using Disc diffusion method

Liquid nutrient agar media and the Petriplates were sterilized by autoclaving at 120°C for 30 minutes. The sterilized petriplates were then labelled with the selected bacterial strains. Under aseptic conditions in the laminar flow hood, 20 ml of agar medium was dispersed into the petriplates labelled with bacterial strains. After solidification of the media, microbial strains were swabbed on the surface of the agar plates separately.

Volume : 4 | Issue : 6 | June 2015 • ISSN No 2277 - 8179

Using sterile forceps, the Antibiotic discs already prepared were placed on the surface of the agar plates. The plates were then incubated at 37°C for 24 - 48 hours. The bacterial culture containing the discs without the addition of the ZnO nano-particles was also taken as the standard and the sterile discs loaded with 20ml of solvent were used as the control for this study. After incubation, all the plates were examined for the presence of evaluated zones of inhibition as a property of anti-microbial activity with intervals of 24hrs and 48hrs. The diameter of the zone was measured in mm and the results were interpreted based on the standard.

3. Results and discussion

3.1. X-ray Diffraction studies

X-ray diffraction pattern recorded ZnO nanopowder is shown in Figure1. It is observed that the prepared samples possess polycrystalline in nature with hexagonal structure with lattice constants (a = 3.0753 Å; c = 5.3273 Å). The diffraction peaks of hexagonal ZnO are found at angles 31.82, 35°.36.32°, 47.6°, 56.7°, 62.9°, 66.4°, 68°, 69.14° corresponding to the lattice planes (002), (100), (101), (102), (110), (103), (200), (112) and (201), respectively. The 'd' values observed in the present work are found to be in close agreement with JCPDS ICDD file for hexagonal ZnO (JCPDS card No.36–1451). Similar behavior is exhibited for ZnO nanoparticles which have been reported earlier [19, 20]. The sizes of the crystallites of the prepared ZnO nanopowder are estimated using FWHM data and Debye-Scherer formula given in Eq (1).

$$D = \frac{0.9\lambda}{\beta \cos\theta} \qquad (1)$$

Where λ is the wavelength of CuK_{α} used, β *is* Full width at Half Maximum of the peak position in radian; θ is Bragg diffraction angle at peak position in degrees. The value of lattice constant 'a' and 'c' are calculated using the below mentioned relation [Eq. (2)]

$$\frac{1}{d^2} = \frac{4}{3}\left[\frac{h^2 + hk + l^2}{a^2}\right] + \frac{l^2}{c^2} \qquad (2)$$

The average crystallite size was found to be in the range 34 nm.

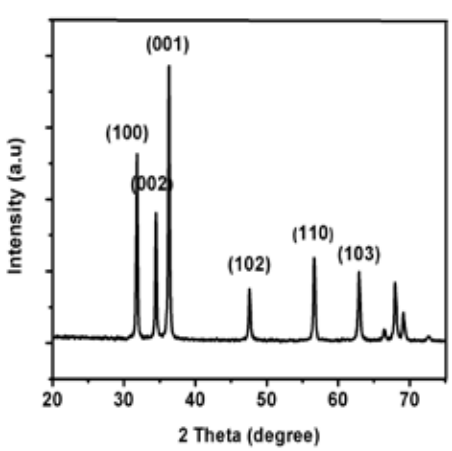


Figure 1 XRD pattern of as formed ZnO nanopowder

3.2 Morphological analysis

The surface morphology of the ZnO nanoparticles was investigated by Scanning Electron Microscope (SEM). The Scanning Electron Microscopy (SEM) was used to analyze the sample morphology and Energy Dispersive Xray Spectroscopy analysis (EDS) gave the chemical composition of the sample. Fig 2(a) exhibited uniform coverage of surface morphology and provide flower shape with different sizes to the synthesized nanoparticles. EDS analysis was used to identify the presence of ZnO nanoparticles in Fig. 2(b).

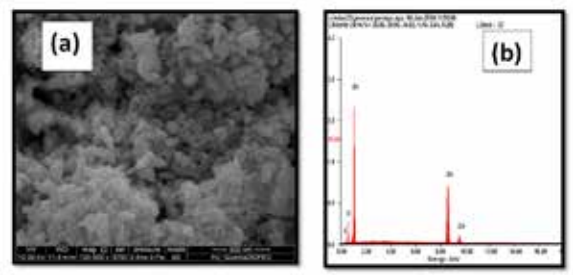


Figure 2. SEM images of ZnO nanopowder

The synthesized powder was also analyzed using a UV–visible spectrophotometer at room temperature to determine the optical properties of the material. A broad band was observed in the spectrum at 362 nm. The optical absorption property of ZnO was studied using UV–vis spectroscopy and the result is displayed in Fig.3. The band gap calculated using $E = hc/\lambda$ formula, ZnO (3.41 eV) is blue shifted as compared to the bulk ZnO (3.2 eV), thus confirming the nanocrystalline nature of the ZnO powder [21, 22].

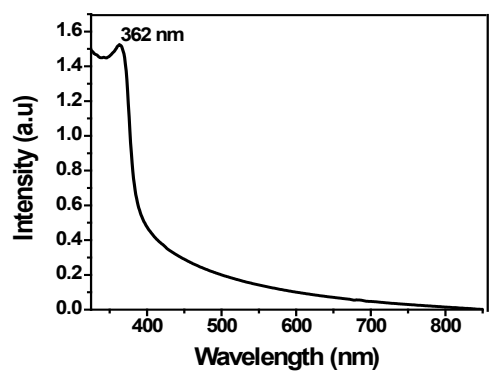


Figure 3. UV- optical absorption spectroscopy for ZnO powder

3.4. FT-IR spectroscopy

FT-IR spectrum of the synthesized ZnO nanopowder showed (fig. 4) the fundamental mode of vibration at 3486 which correspond to the O-H stretching vibration, 2917.57 and 2374 which corresponds to C-H stretching vibration and 1465 corresponds to C=O asymmetric C=O stretching vibration. 1437 corresponds to C=O symmetric stretching vibration. The absorption at 900.4 and 848.36 cm-1 is due to the formation of tetrahedral coordination of Zn. The bond at 1016.3 cm-1 is due to the C-O stretching vibration [21, 22].

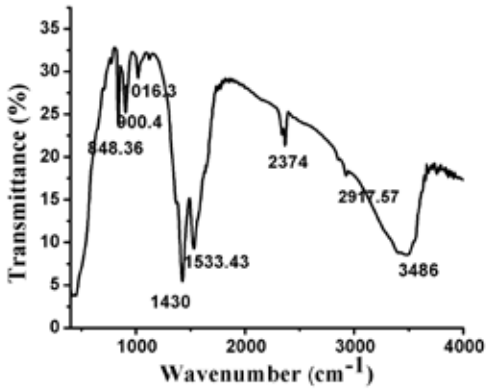


Figure 4. FTIR spectroscopy for ZnO powder

3.5. Photoluminescence spectroscopy

Photoluminescence technique is suitable to determine the crystalline quality and presence of impurities in the materials, as well as, exciton fine structure. In Fig. 5, a strongest peak around 360 and 393 nm for ZnO nanopowder is the near band edge emission (UV emission) due to the free-exciton recombination [23]. The presence of the relatively weak peaks at 410 and 436 nm can be attributed to defects such as zinc vacancies and anti-sites zinc–oxygen. Similar peaks around 393 and 410 were reported by Singh et al. [24] for the ZnO nanoparticles. The blue emission at 490 nm is attributed to intrinsic defects such as oxygen and zinc interstitials. The peak around 520 nm is the green emission and usually due to surface defects, oxygen vacancies which can trap the electrons from the valence band and act as luminescent center. Generally, a green-yellow emission is observed in PL spectra, due to recombination of photo generated holes with singly ionized charge state of specific defect. Similar type of PL emission has been reported by Hwangbo et al. [25] for sol–gel-derived ZnO thin films

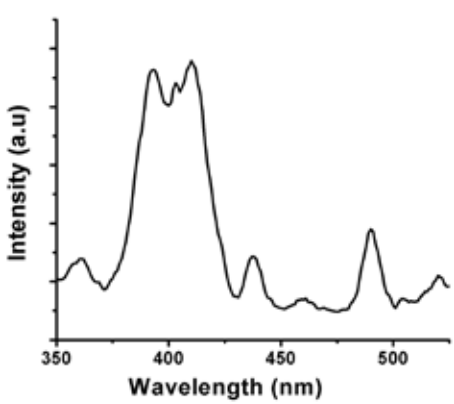


Figure. 5: PL spectra of the ZnO nanopowder

3.5. Antibacterial effect

The antibacterial activity of ZnO nanopowder was assayed in vitro by agar disc diffusion method against 6 bacterial pathogenic species. Table - 1 summarizes the microbial growth inhibition by the ZnO nanopowder. The maximum antibacterial activity was shown on the *Escherichia coli* followed by *Klebsiella pneumonia*, *Pseudomonas aeroginosa*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Streptococcus agalactiae*, In the present investigation ZnO nano-particles have high antibacterial activity against *Escherichia coli* was investigated. The extent of inhibitory activity on the culture of pathogenic bacterial strains for 48 hours has been shown in Fig.5A to 3F.

Among the six different bacterial pathogenic strains ZnO nanopowder showed highest impact on Escherichia coli. They are facultative anaerobes constitute about 0.1% of gut flora, and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. As an indicator organisms E.coli are used to measure such things as potential fecal contamination of environmental samples.

The presence of coliform bacteria, such as *E. coli*, in surface water is a common indicator of faecal contamination. Bacterial infections are usually treated with antibiotics. However, the antibiotic sensitivities of different strains of *Escherichia coli* vary widely. As gram negative organisms, they are resistant to many antibiotics that are effective against gram-positive organisms. Antibiotics which may be used to treat *E. coli* infection include amoxicillin, as well as other semi synthetic penicillins [26, 27]. Hence the present investigation stated that Zno nanoparticles will be of more useful in the control of the pathogenic strain of *E.coli*.

The antimicrobial properties of the ZnO nanopowder evaluated against both Cattle and poultry pathogens under normal lighting condition.The antibacterial activity of ZnO were assayed by agar disc diffusion method against 6 bacterial pathogenic species. Table - 1 summarizes the bacterial growth inhibition by the ZnO. The diameters of the zone of inhibition around the membranes after 24 hours were measured to be 12 mm, 14 mm, 10 mm, 3 mm, 5mm and 5mm for *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeroginosa, Streptococcus agalactiae*, , *Salmonella typhimurium* and *Staphylococcus auereus* respectively. After 48 hours, the diameters of the zone of inhibition increased (fig. 7). The maximum antibacterial activity was shown on the *Escherichia coli* (16mm) and *Klebsiella pneumonia* (16 mm) followed by *Pseudomonas aeroginosa* (12mm), *Staphylococcus auereus* (3 mm), *Salmonella typhimurium* (6.5 mm) and *Streptococcus agalactiae* (7 mm). In the present investigation ZnO have high antibacterial activity against *Escherichia coli* was investigated. The extent of inhibitory activity on the culture of pathogenic bacterial strains has been shown in Fig 7.

Sl.No.	Name of bacteria (ZnO Control)	Zone of inhibition(mm) (Diameter)	
		24 hrs	48 hrs
1	E.Coli	12	16
2	Kleb. pneumonia	14	16
3	*Pseud. aeruginosa*	10	13
4	Staph. auereus	3	3
5	Salm. typhimurium	5	6.5
6	Strep. agalactiae	5	7



Figure 6. Zone of Inhibition by ZnO Nano-powder on six different animal bacterial pathogens for 48hours

Volume : 4 | Issue : 6 | June 2015 • ISSN No 2277 - 8179

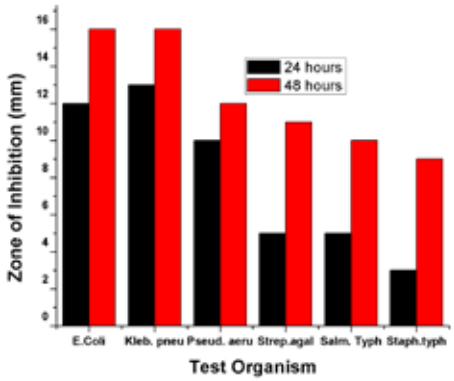


Figure. 7 Inhibition zones caused by ZnO nanopowders against different bacterias.

Li *et al.* (2009) [28] reported the antibacterial effect of ZnO powder coated PVC film against Gram-positive and Gram-negative bacteria. The larger surface area and higher concentration are responsible for the antibacterial activity of ZnO nanopowder [29]. The previously reports demonstrated that the generation of H_2O_2 from ZnO leads to the penetration of particles into the cell membrane of bacteria leads to the formation of injuries and finally the death of bacterium was occurred [30]. Conversely, the electrostatic interaction between bacterial cell surface and nanop0wder may be one of the reasons for the inhibition of growth [31]. In another study, ZnO nanopowder were observed to have impact on the DNA damage. Collectively, a huge number of researchers have studied the effect of nanopowder on the gram-negative and gram-positive bacteria such as *E.coli* and *Staphylococcus aureus* as well as human immune cells. But the results of these studies have demonstrated that the nanopowder had harmful effects on bactericidal strains [32]. Based upon the above possible phenomena, our present study reports the growth inhibition of *E. coli, Streptococcus agalactiae* and *Pseudomonas aeruginosa* was obtained by damage the cell membrane through penetration of ZnO nanopowder.

Conclusion

A simple, rapid inexpensive method has been developed to prepare ZnO nanopowder. The PXRD pattern shows sharp and intense peaks, indicating the existence of crystallite structures of size 34 nm even at room temperature and confirms the hexagonal wurtzite phase. The morphological details obtained by SEM show the presence of agglomerated and having flower shape. The antibacterial efficiency of ZnO nanopowder against different cattle and poultry pathogenic bacterials stains were carried in the present study using disc diffusion method. All the six bacterial strains were dangerous to livestock population which causes considerable mortality in livestock population leading to economic loss to humans. The results proved that the ZnO nanoparticles have potential to be used as an antimicrobial agent. ZnO Nanopowder have showed highest impact on the common animal pathogen *E.coli* (16mm) and hence it can be employed to control this bacterial strain Results prove that ZnO show more enhanced biocidal activity against various pathogens and is suggested ZnO could be used effectively in agricultural food safety applications and future medical concerns.

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