https://doi.org/10.30799/jnst.138.18040410 J. Nanosci. Tech. - Volume 4 Issue 4 (2018) 439–442

ISSN: 2455-0191

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Synthesis, Characterization and Antibacterial Activity of Zinc Oxide Nanoparticles

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ARTICLE DETAILS

Article history:
Received 04 July 2018
Accepted 14 July 2018
Available online 22 July 2018

Keywords:
Zinc Oxide Nanoparticles
Spectral Characterizations
Antibacterial Activity

ABSTRACT

Zinc oxide nanoparticles were synthesized by a chemical method and characterized by various analytical techniques such as UV-visible spectroscopy, SEM-EDX, XRD, and FTIR. X-ray diffraction and SEM analysis confirmed the formation of well- dispersed zinc oxide nanoparticles with average particle size 22 nm as well as revealed their spherical structure. Chemically synthesized nanoparticles were found to exhibit antibacterial activity against bacterial strains *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* using agar well diffusion method of analysis.

1. Introduction

Nanotechnology is the technology to control a matter of an atomic and molecular scale. It has extraordinary potential to change the lives by improving existing products and enabling new ones. It facilitates the development of new materials in the 1-100 nm range, comparable to the size range of biological molecules and structures [1]. Nanoparticles are used as manipulation, sensing, and detection of biological structures and systems. The principal factors which make nanomaterials different from their bulk counterparts include an increase in their relative surface area and quantum effects, which affect their physical and chemical properties [2] and due to the high surface area to volume ratio, it develops as novel antimicrobial agents and flow enthusiasm to the researchers because of the developing microbial resistances against metal ions, anti-toxins and the development of resistant strains [3]. Due to the rapid development of nanotechnology, nanomaterials with various shapes and diameters have been prepared and used in some industrial products and commodities [4-6].

Among several nanoparticles, ZnO nanoparticles (n-ZnO) have received more attention. Zinc oxide nanoparticles (ZnO-NPs) are common nanoparticles and widely used in many fields such as sunscreen products, cosmetics, pigments, industrial coatings, plastic additives, semiconductors, textiles, and antibacterial agents [7]. It also exhibits antibacterial activities when the particle is in the nanometer range, at that point ZnO Nps can connect with the bacterial surface as well as with the bacterial core, and consequently exhibits bactericidal components [8].

Due to numerous applications of nanoparticles, research on related to preparation and to know the properties of nanoparticles have become more attention in the past several years. Production of nanoparticles can be achieved through conventional chemical methods [9] and physical methods [10]. When comparing with physical method chemical method used for synthesizing the nanoparticles are simple and more effective. Furthermore, ZnO nanoparticles effectively resist microorganisms [11]. The work related to the antibacterial activity of ZnO nanoparticles against with three strains pathogenic bacteria such as Gram –ve *Escherichia coli, Pseudomonas aeruginosa* and Gram +ve *Staphylococcus aureus* is totally wanting. Hence the present study deals with synthesis of zinc oxide nanoparticles and its antibacterial activity.

2. Experimental Methods

Zinc acetate and sodium hydroxide were purchased from Nice chemicals, India. All the reagents used for the synthesis zinc oxide nanoparticles were analytical grade and used without further purification. All the glass wares were washed thrice with deionized water and dried before use.

2.1 Synthesis of Zinc Oxide Nanoparticles

Synthesis of zinc oxide nanoparticles was carried by simple precipitation method. For this study, 0.5 M of zinc acetate ($Zn(CH_3COO)_2.2H_2O$) was dissolved in 100 mL of distilled water and 1 M of sodium hydroxide were also dissolved in 100 mL of distilled water. Precipitation was done by mixing of 1 M NaOH which is to be added in a dropwise to the 0.5 M of zinc acetate solution under vigorous stirring. The process continued until the appearance of a milky white precipitate. During this precipitating process, pH was increased from 7 to 14. Following the precipitation, the solution was centrifuged at 3000 rpm for 10 min and washed for several times with distilled water and ethanol to remove the by-products. The supernatant was then removed and the pellet was dried. After drying, the precipitate was calcined in a muffle furnace at 300 °C for 3 h and ZnO nanopowder was ground into fine powder.

2.2 Characterization of Zinc Oxide Nanoparticles

The chemically synthesized zinc oxide nanoparticles were characterized by UV-Vis spectroscopy using automated spectrometer Spectro UV-Vis double beam DUV 3500, scanning electron microscopy (SEM) using an LEO 1455 VP equipped with energy dispersive. The possible functional groups of ZnO Nps were analyzed by using Fourier transform infrared spectroscopy (FTIR) analysis with an instrument JASCO (FTIR-6200) spectrum. The X-ray diffraction was carried out by an X-ray diffractometer (Shimadzu XRD-6000, Japan) for the crystallographic structural analysis.

2.3 Antibacterial Activity

2.3.1 Preparation of Inoculums

One gram-positive *Staphylococcus aureus* and two gram-negative *Escherichia coli, Pseudomonas aeruginosa* bacterial strains were used for this study. Then the active cultures were prepared by transferring a loopful of culture from the stock to test tubes of nutrient broth that were incubated for 24 h at 37 °C and used as inoculums for antibacterial activity.

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https://doi.org/10.30799/jnst.138.18040410

Cite this Article as: V. Keerthika, A. Ananth, M.R. Rajan, Synthesis, characterization and antibacterial activity of zinc oxide nanoparticles, J. Nanosci. Tech. 4(4) (2018) 439–442.

2.3.2 Agar Well Diffusion Method

Nutrient agar plates were swabbed on three axes with the sterile cotton-tipped swab, which was dipped in the freshly prepared diluted culture. Sterile cork borer was used to make the two 6 mm holes aseptically in the agar plate. ZnO Nps were prepared by diluting 10 mg of dry ZnO Nps with 100 mL sterile distilled water and dispersed in water using ultrasonicator for 20 minutes. One hole was filled with ZnO Nps, one antibiotic disc and one well filled with distilled water which serves as a control. Then plates were incubated at 30 °C for 24 h. After incubation, the plates were observed for the zone of inhibition which was measured in terms of diameter.

3. Results and Discussion

3.1 Synthesis of Zinc Oxide Nanoparticles

As sodium hydroxide (NaOH) was added to the zinc acetate($Zn(CH_3COO)_2.2H_2O$), it is found to be colour changes from colorless to milky white precipitate (Fig. 1) and this precipitate confirms the formation of zinc oxide nanoparticles.

$$Zn(CH_3COO)_2.2H_2O + 2NaOH \rightarrow Zn(OH)_2 + 2H_2O + 2CH_3COONa$$
$$Zn(OH)_2 + 2H_2O \leftrightarrow Zn_2^+ + 2OH^- + 2H_2O \leftrightarrow [Zn(OH)_4]^{2-}$$
$$[Zn(OH)_4]^{2-} \leftrightarrow ZnO_2^{-2} + 2H_2O$$
$$ZnO_2^{-2} + 2H_2O \leftrightarrow ZnO + 2OH^-.$$

Fig. 1 Synthesis of Zinc Oxide Nanoparticles

3.2 Characterization of Zinc Oxide Nanoparticles

3.2.1 UV-Vis Analysis

The primary characterization of chemically synthesized nanoparticles was done with UV-Vis spectroscopy which is a very useful and reliable technique. Zinc oxide Nps have unique optical properties which make them strongly interact with specific wavelengths of light. In addition, UV-VIS spectroscopy is simple, easy, fast, required a short period of time for measurement, and to characterize the colloidal suspensions [12]. UV-Vis spectroscopy analysis of chemically synthesized ZnO Nps analyzed between the range 300 – 500 nm (Fig. 2) and showed high peak absorbance spectrum at 335nm. Satyanarayana et al. [13] also reported a peak at 320 nm for zinc oxide nanoparticles.

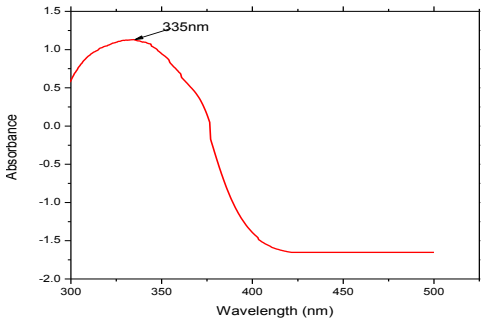

Fig. 2 UV-Vis analysis of zinc oxide nanoparticles

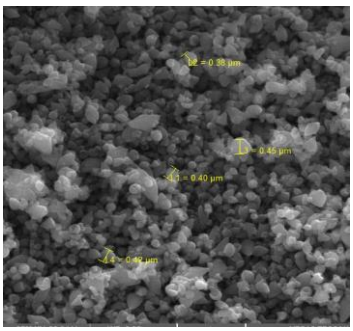

Fig. 3 SEM image of zinc oxide nanoparticles

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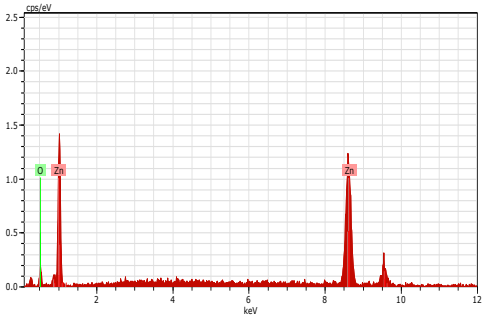


Fig. 4 EDAX analysis of zinc oxide nanoparticles

3.2.2 SEM-EDAX Analysis

SEM analysis was conducted in order to examine the morphology and structure of the chemically synthesized ZnO nanoparticles. This reveals that the obtained nanoparticles are uniform spherical in shape and shown in Fig. 3. It can be also used for predicting the antimicrobial activity of ZnO nanoparticles. Nie et al. [14] reported that the results from scanning electron microscopy indicated the presence of nanoparticles in growing medium may cause damages to the *E. coli* cell membrane. Fig. 4 indicates the high purity and chemical composition of ZnO nanoparticles which are confirmed by EDX analysis.

3.2.3 X-Ray Diffraction Analysis (XRD)

The crystalline structure of chemically synthesized zinc oxide nanoparticle was obtained using XRD. Dorofeey et al. [15] reported that XRD analysis is important and act as good potential techniques for nanostructures examination because it yields information about the substructure and sizes of crystallites of the materials. Fig. 5 shows that ZnO nanoparticle was highly crystalline and all diffraction peaks are well indexed 31.687°, 34.339°, 36.172°, 47.453°, 56.507°, 62.768°, 66.281°, 67.856°, 68.981°, 76.908°, 92.670°, 95.314° and 98.472° which corresponds to 100, 002, 101, 102, 110, 103, 200, 112, 201, 202, 210, 211and 114 crystal plane respectively (JCPDS 36-1451). Giri et al. [16] reported similar peaks for zinc oxide nanoparticle. The average crystalline size of ZnO nanoparticle is 22nm.The crystallite domain size of XRD peaks was calculated from the width of resulted peaks, confirming that they were free from non-uniform strains by adopting Scherrer's formula, $D = 0.94\lambda/\beta\cos\theta$, where, D = average crystallite domain size, λ = X-ray wavelength, β = full width at half maximum (FWHM), and θ = diffraction angle.

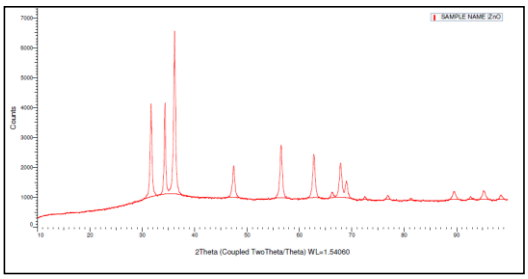


Fig. 5 XRD analysis of zinc oxide nanoparticle

3.2.4 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is used to measure the vibration modes of functional groups of molecules and is sensitive to molecular structure, conformation, and environment. Therefore, in the current study, it is possible to directly relate the intensities of the absorption bands to the concentration of the corresponding functional groups. FTIR spectroscopy was analyzed in the range of 4000 – 400 cm^{-1}. The FTIR spectra of chemically synthesized zinc oxide nanoparticles were analyzed for knowing the possible functional groups (Fig. 6). This FTIR spectrum shows the purity of ZnO nanoparticles, the peak at 402.0 cm^{-1} is the characteristic absorption of Zn-O bond and the broad absorption peak at 3459.6 cm^{-1} can be attributed to the characteristic absorption of hydroxyl groups (Table 1). Similarly [17] reported that ZnO nanoparticles exhibit a rather broad and moderately strong band at 495 cm^{-1}, owing to Zn-O vibrational mode. The band at 3445 cm^{-1} corresponds to O-H mode of adsorbed moisture in the annealed sample. The stretching C-O vibration is observed at 1530 cm^{-1}. The bands at 1625 and 2363 cm^{-1} are due to C=O and CO_2 groups. Sharma et al. [18] applied the FTIR technique to characterize ZnO Nps and showed a standard peak of zinc oxide is around 464 cm^{-1}.

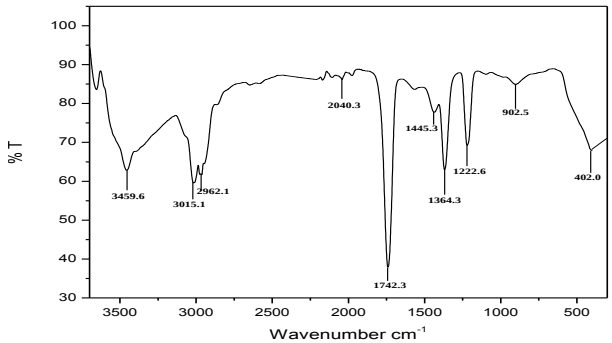


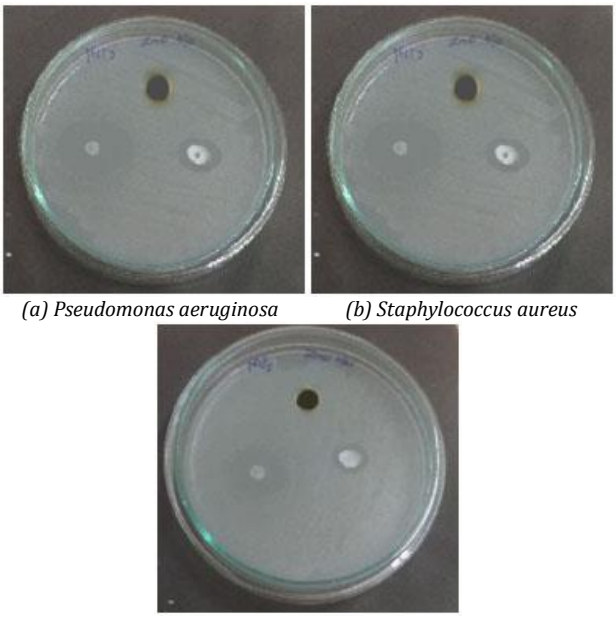
Fig. 6 FT-IR analysis of zinc oxide nanoparticles

Table 1 Functional group assignments of FT-IR characterization

S. No.	Wave number ZnO Nps (Cm^{-1})	Spectral assignments
1	3459.6	O-H Stretching vibration, broad
2	3015.1	=C-H Stretching vibration
3	2962.1	CH_3 and CH_2 Stretching vibration
4	2040.3	CC Stretching vibration
5	1742.3	C=O Stretching vibration
6	1445.3	C-O Stretching mode, carbonate
7	1364.3	C-H bond bending
8	1222.6	C-H Deformation vibration
9	902.5	CH_2 Deformation vibration
10	402	Zn-O Stretching vibration, broad

Table 2 Antibacterial activity of ZnO Nps against pathogens

Pathogens	Zone of inhibition (mm)	
	ZnO Nps	Antibiotic
Pseudomonas aeruginosa	13	20
Staphylococcus aureus	15	22
Escherichia coli	10	18



(a) Pseudomonas aeruginosa *(b) Staphylococcus aureus*

(c) Escherichia coli

A) *Antibiotic* B) *ZnO Nps* C) *Control*

Fig. 7 Zone of Inhibition of ZnO Nps against three bacterial strains

3.3 Antibacterial Activity of Zinc Oxide Nanoparticles

The antibacterial activity of chemically synthesized zinc oxide nanoparticles against three strains of pathogenic bacteria such as *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus* was done by using well diffusion method. Antibacterial effect was measured by observing the zone of inhibition. The presence of an inhibition zone clearly indicated the antibacterial effect of ZnO nanoparticles as presented in Table 2. This assays revealed that the gram-negative bacteria are sensitive to the ZnO NPs, while it shows resistance to the synthetic antibiotic (Fig. 7). Sivakumar and Senthilkumar [19] observed the similar antibacterial activity of zinc oxide nanoparticles. Literature revealed that the electrostatic interactions between ZnO NPs and cell walls resulting in destroying bacterial cell integrity [20], the liberation of antimicrobial Zn^{2+} ions [21] which is related to an accumulation of ZnO NPs into the bacterial cells [22]. Mekala and Rajan [23] reported that CuO nanoparticles attach to the surface of the cell membrane disturbs its function and penetrates directly with the bacterial outer membrane and release CuO ions and smaller sized NPs can enter the mitochondria of cells through various pathways and thereby induce oxidative stress and cell death via apoptosis [24]. Thus ZnO NP may contort and harm bacterial cell layer, causing spillage of intracellular substance promoting cell death [25].

4. Conclusion

ZnO nanoparticles have been prepared using a wet chemical method. The structural characterization of synthesized nanoparticles are crystalline in structure and their diameter was around 22 nm confirmed by XRD. The synthesized ZnO nanoparticles exhibit the UV absorption peak at 335 nm. In FT-IR spectroscopy pure ZnO nanoparticles show stretching vibrations at 402 cm^{-1}. The chemically synthesized ZnO Nps exhibit significant antibacterial activity against all the three bacterial strains, i.e., gram -ve *E.coli, Pseudomonas aeruginosa* and gram +ve bacteria *Staphylococcus aureus* and show a significant zone of inhibition to ZnO Nps compared to the positive control penicillin. This study proves that zinc oxide nanoparticles possess good antibacterial activity.

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https://doi.org/10.30799/jnst.138.18040410

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