

## Pharmacological studies of zinc oxide nanoparticles

Veronica Deekala<sup>1</sup>, Bonige Kishore Babu<sup>2\*</sup> & Rameshrajru Rudraraju<sup>1\*</sup>

<sup>1</sup>Department of Chemistry, Acharya Nagarjuna University, Guntur- 522 510, Andhra Pradesh, India

<sup>2</sup>Department of Engineering Chemistry, Andhra University, Vishakapatnam- 530 001, Andhra Pradesh, India

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In this paper, we focused on the synthesis and characterization of zinc oxide nanoparticles by using a chemical method. The characterization of Zinc oxide Nanoparticles are determined by different spectroscopic techniques such as UV-Visible, DRS, FTIR, FESEM, and EDX. Further, the antimicrobial activity and cytotoxic studies also performed towards different bacterial species like *Bacillus subtilis*, *Escherichia coli*, fungi like *Aspergillus niger* and MCF-7 Breast Cancer cell line.

**Keywords:** *Aspergillus niger*, *Bacillus subtilis*, *Escherichia coli*, Nanoparticles, Zinc oxide

Nanoparticles are being synthesized globally owing to various exciting and unique properties, which facilitate their exploitation in completely unrelated fields, such as, nanodiagnosics<sup>1-3</sup>, nanomedicine<sup>4</sup> and antimicrobials<sup>5</sup>. In the extant study, it also covers about luminescence<sup>6</sup>, photocatalytic potential<sup>7</sup> and photodiode response<sup>8</sup>. These zinc oxide Nanoparticles are biologically evident as it offers easy fabrication, biosafe, biocompatible and nontoxic<sup>9,10</sup>. Additionally, as per the US Food and Drug Administration, ZnO with other four zinc compounds have been listed as generally recognized as safe [1922]. Various chemical methods have been proposed for the synthesis of ZnO NPs, such as the reaction of zinc with alcohol, vapor transport, hydrothermal synthesis, precipitation method, *etc.* However, these methods suffer various disadvantages due to the involvement of high temperature and pressure conditions and the use of toxic chemicals<sup>11,12</sup>. In view of this, various metal and metal oxide nanoparticles have been successfully synthesized using chemical methods<sup>13</sup> including some rare higher oxides such as Cr<sub>2</sub>O<sub>3</sub>, Eu<sub>2</sub>O<sub>3</sub> and Sm<sub>2</sub>O<sub>3</sub><sup>14</sup>.

Recently, ZnO NPs have been used in food packaging materials and various matrices and methods for incorporation of ZnO into those matrices have been reported. ZnO is incorporated into the packaging matrix, free to interact with the food materials offering preservative effects<sup>15,16</sup>. Presently, ZnO NPs have found application in sunscreens, paints, and coatings as they are transparent to visible

light and offer high UV absorption<sup>17,18</sup> and are also being used as an ingredient in antibacterial creams, ointments and lotions, self-cleaning glass, ceramics and deodorants<sup>19</sup>. ZnO nanoparticles have been lately tested for their antimicrobial potential and seem to possess both antibacterial and antifungal potential. They are active against both Gram-positive and Gram-negative bacteria and also show considerable activity against more resistant bacteria spores<sup>20,21</sup>. It was also observed that doping of ZnO NPs with other metals such as gold, silver, chromium, *etc.* improved the antimicrobial activity of ZnO NPs<sup>22</sup>. Also, the inhibitory effects of ZnO nanosuspension are correlated with their size and concentration, with smaller particles offering better inhibitions in higher concentrations<sup>23</sup>.

Majorly researched substrate for the synthesis of ZnO NPs is zinc nitrate. This is, to the best of our knowledge, the first study reporting the synthesis of zinc oxide nanoparticles using chemical method and zinc nitrate.

### Materials and Methods

#### Experimental

##### Reagents

Chemicals are procured from renowned companies like Sigma Aldrich, Molychem and used without further purification. Experimental Zinc Nitrate (Zn(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O), Sodium hydroxide and Double distilled water was used as the solvent throughout the experiment. Chemicals are procured from renowned companies like Sigma Aldrich, Molychem and used without further purification. Experimental Zinc

Nitrate ( $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ), Sodium hydroxide and Double distilled water was used as the solvent throughout the experiment.

#### Synthesis of Zinc oxide Nanoparticles

Zinc nitrate hexahydrate  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  and sodium hydroxide NaOH were each dissolved separately in deionized water to form the liquid media of the desired concentrations of 0.05 M (12.85 g/500 mL) and 0.1 M (2 g/500 mL) for sample A and B, respectively, the ratio of the concentrations was 1:1 ( $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ : NaOH). The Zinc nitrate hexahydrate was slowly added drop-wise to NaOH solutions under vigorous stirring at room temperature, forming transparent white solutions, then inserted into an electrical oven at  $90^\circ\text{C}$  for 2 h. These solutions were reacted to produce zinc oxide precipitates. Following the precipitation, the solution was centrifuged at 3000 rpm for 30 min. The supernatant was then removed, and the precipitation which contains ZnO was obtained. Finally, zinc oxide was ground with mortar to be shaped into powder.

#### Characterisation

The synthesized zinc oxide nanoparticles were characterized by using a UV- VISIBLE DRS in the wavelength range between 200-800 nm. The FTIR absorption spectra were recorded on a Perkin-Elmer GX FT-IR system used to obtain  $16\text{ cm}^{-1}$  resolution spectra in the range 400 to  $4000\text{ cm}^{-1}$  (absorbance mode).

The crystalline structure of the zinc oxide nanoparticles was measured by using a Bruker D8 Advance X-ray diffract meter with  $\text{CuK}\alpha$  radiation of wavelength  $\lambda = 1.54056\text{ \AA}$ . The X-ray diffraction (XRD) measurements were carried out in the locked coupled mode in the  $2\theta$  range of  $20$  to  $80^\circ$ .

The surface morphology and composition of zinc oxide nanoparticles were investigated by FESEM (Field Emission Scanning Electron Microscopy) developed by Carl Zeiss. An accelerating voltage of 15 to 19 keV and probe current of  $\sim 800\text{ pA}$ .

#### Optical characterization

It was carried out by measuring the diffuse reflectance spectroscopy in the UV-Vis range. The spectrum was taken in the range of 200-700 nm. Figure 1 shows the diffused reflectance spectra (absorbance as a function of wavelength). The excitation absorption is at about 362 nm. The optical band gap  $E_g$  of the nanoparticles was calculated from tauc plot as shown in (Fig. 2) the presence of a single

slope in the plot suggests that the films have direct and allowed the transition. For such transition we have

$$(\alpha h\nu)^2 = A(h\nu - E_g)^n \dots \quad \dots (1)$$

where  $\alpha$  is absorption coefficient,  $h\nu$  is photon energy,  $E_g$  is optical band gap,  $n$  is 1 for direct transition &  $A$  is a constant. Figure 2 shows the band gap energy is obtained by extrapolating the straight-line portion of the plot to zero absorption coefficient. The band gap value of ZnO nanoparticles is found to be 3.20 eV. This redshift of the band gap energy is due to agglomeration of the nanoparticles into larger particles are reported by various authors in different kinds of literature.

#### Fourier-transform infrared spectroscopy (FTIR)

Figure 3 shows FTIR spectra of ZnO nanoparticles. Infrared studies were carried out to ascertain the

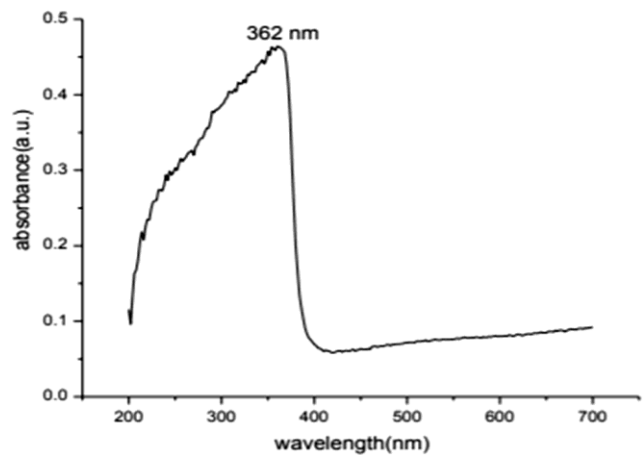


Fig. 1 — UV-visible of synthesized ZnO nanoparticles

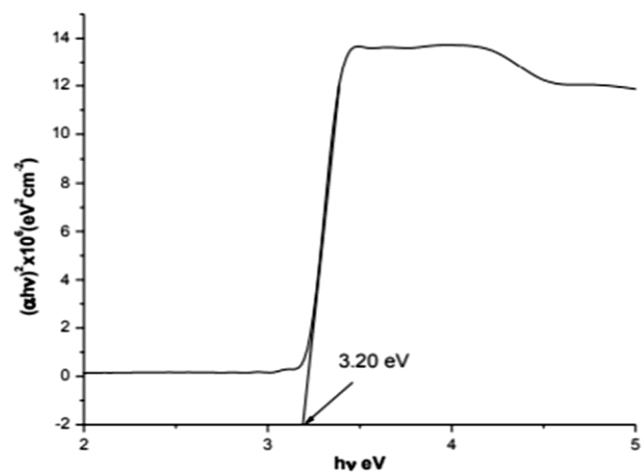


Fig. 2 — Tauc plot for determination of band gap

purity and nature of the metal nanoparticles. Metal oxides generally give absorption bands in fingerprint region *i.e.* below  $1000\text{ cm}^{-1}$  arising from inter-atomic vibrations. The peak observed at  $3461.10$  and  $1384.08\text{ cm}^{-1}$  are may be due to O-H stretching and deformation, respectively, assigned to the water adsorption on the metal surface. The peaks at  $1637.08$ ,  $623.10\text{ cm}^{-1}$  are corresponding to ZnO stretching and deformation vibration, respectively (Table 1). The metal-oxygen frequencies observed for the respective metal oxides are by literature values<sup>25-29</sup> reported similar FTIR spectra observed of zinc oxide nanoparticles in their investigation.

#### X-ray diffraction analysis

Figure 4 shows the XRD diffraction pattern of ZnO nanoparticles. The peaks are indexed as  $26.85^\circ$  (100),  $30.63^\circ$  (002),  $33.29^\circ$  (101),  $44.96^\circ$  (102),  $56.37^\circ$  (110),  $58.32^\circ$  (103),  $60.25^\circ$  (200),  $67.52^\circ$  (112),  $69.56^\circ$  (201) and  $76.23^\circ$  (202), respectively. All diffraction peaks of the sample correspond to the characteristic hexagonal wurtzite structure of zinc oxide nanoparticles<sup>30-32</sup>. Similar, X-ray diffraction pattern was reported by<sup>33-35</sup>. The average particle size of ZnO nanoparticles is found to be  $10.0\text{ nm}$  using The Scherrer equation. Diffraction pattern corresponding to impurities are found to be absent. This proves that pure ZnO nanoparticles were as synthesized.

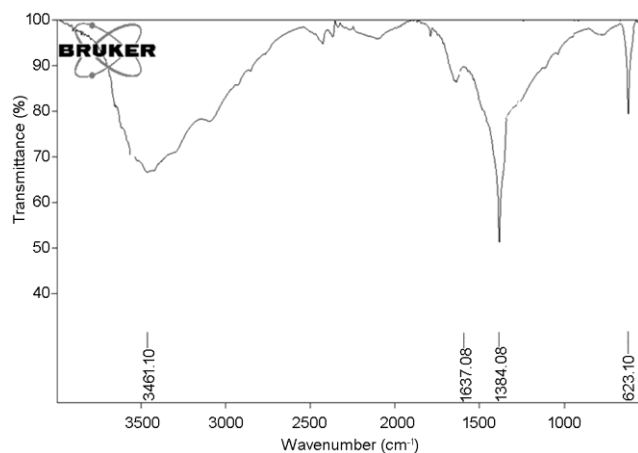


Fig. 3 — Shows the FTIR spectrum of the zinc oxide nanoparticles

Table 1 — Comparison of Vibrational modes of CuO nanoparticle

S. No.	CuO ( $\text{cm}^{-1}$ )	Vibrational modes
1	3461.10	OH
2	1384.08	OH deformation
3	623.10	Stretching of CuO
4	1637.08	C-H

Interplanar d-spacing was calculated using Bragg's Law equation (Table 2):

$$2d \sin\theta = n \lambda \dots \quad \dots (2)$$

where  $\theta$  is Bragg's angle of diffraction,  $\lambda$  is X-ray wavelength, *i.e.*  $1.5406\text{ \AA}$  and  $n = 1$ . Further, particle size was calculated from the intense peak corresponding to (101) plane using Debye-Scherrer formula

$$D = 0.89\lambda / \beta \cos\theta \dots \quad \dots (3)$$

where  $0.89 =$  Scherrer's constant,  $\lambda =$  X-ray wavelength ( $1.5406\text{ \AA}$ ),  $\beta =$  FWHM (Full Width at Half Maximum) of the peak located at  $2\theta = 36.27$  and  $\theta =$  Bragg's angle of diffraction. The value of particle size was found to be  $16.78\text{ nm}$ .

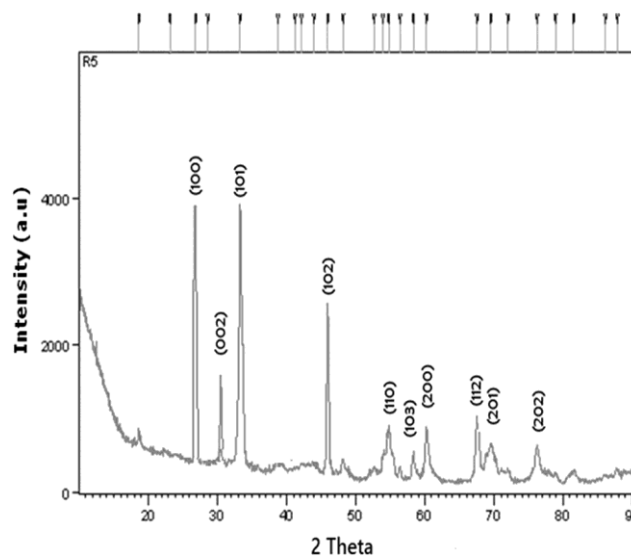


Fig. 4 — XRD pattern of ZnO nanoparticles

Table 2 — d-spacing calculations for ZnO NPs

$2\theta$	$\theta$	$\sin\theta$	$d(\text{\AA})$	Nm	Hkl
26.87	13.43	0.232	3.31	0.77	100
30.57	15.28	0.263	2.92	0.85	102
33.57	16.78	0.288	2.66	0.76	101
36.27	18.13	0.311	2.47	0.86	102
55.07	27.53	0.462	1.66	0.73	110
58.39	29.19	0.487	1.57	0.75	103
60.93	30.6	0.506	1.51	0.72	200
67.93	33.96	0.558	1.37	0.66	112
69.78	34.89	0.571	1.34	0.73	201
76.33	38.16	0.617	1.24	0.71	202

FE-SEM image (Fig. 5). shows the surface morphology and particle size of the synthesized ZnO nanoparticles. It is clear from the images that the size of the ZnO nanoparticles is ranging from 24-26 nm. The obtained products are composed of near flower-shaped morphology with an average size in the range of 50 nm.

#### Scanning electron microscope (SEM)

SEM image (Fig. 5) shows the surface morphology and particle size of the synthesized ZnO nanoparticles. It is clear from the images that the size of the ZnO nanoparticles is ranging from 24-26 nm. The obtained products are composed of nearly flower shaped morphology with average size in the range of 50 nm.

#### The energy dispersive X-ray diffractive (EDX)

The Energy dispersive X-ray Diffractive (EDX) study was carried out for the synthesized ZnO nanoparticles (Fig. 6) to know about the elemental composition. EDX confirms the presence of element zinc and oxygen signals of zinc oxide nanoparticles and this analysis showed the peaks that corresponded to the optical absorption of the produced nanoparticles. The elemental analysis of the nanoparticles yields 63.27% of zinc and 36.73% of oxygen which proves that the produced nanoparticles is in its highest purified form and was agreed with the earlier studies<sup>36,37</sup>.

Journal of Sangeetha N, Kuppusamy KA Extracellular of zinc oxide nanoparticle using seaweeds of Gulf of Mannar, India.

#### Antimicrobial Screening of ZnO

The nanoparticles are screened *in vitro* for antibacterial activity against *E.coli*, *B. subtilis* and antifungal activity against *A. niger* by Agar-well

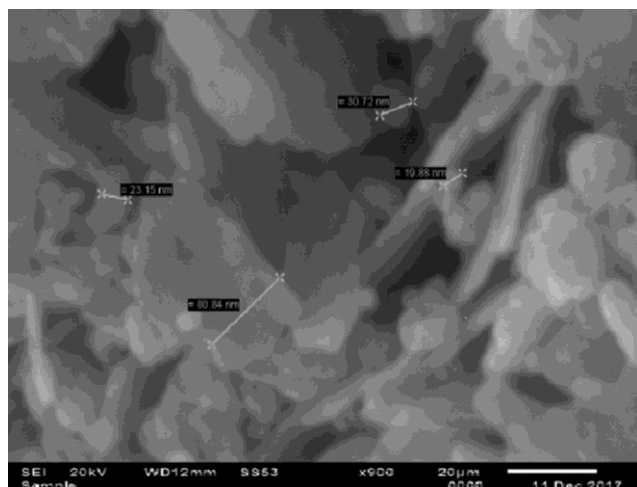


Fig. 5 — SEM image of ZnO nanoparticles

diffusion method. The antibacterial and antifungal activities of ZnO nanoparticles are listed in (Table 3).

ZnO nanoparticle shows antifungal activity against fungal organisms like *Aspergillus Niger* and antibacterial activity against *Bacillus subtilis* and *Escherichia coli* (Fig. 7). The obtained inhibition

Table 3 — Antimicrobial activities of ZnO nanoparticles

Bacteria	Inhibition zone (mm)
<i>Escherichia coli</i>	20
<i>Bacillus subtilis</i>	21
Fungi	Inhibition zone (mm)
<i>Aspergillus Niger</i>	12

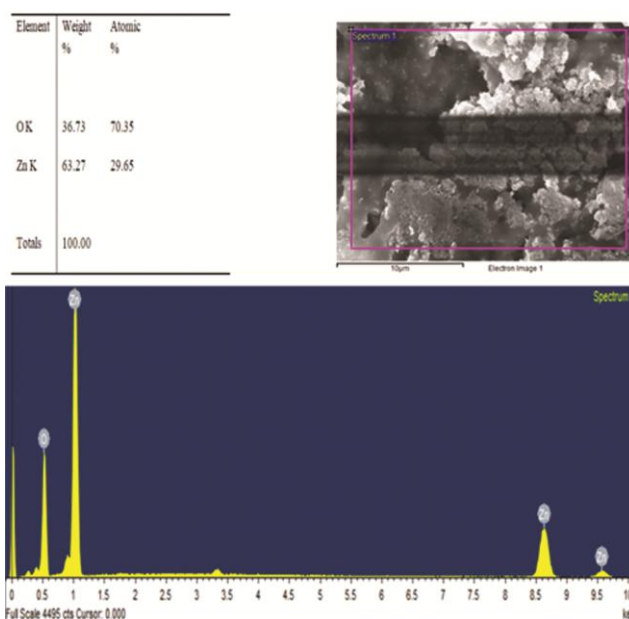


Fig. 6 — EDX of ZnO nanoparticles

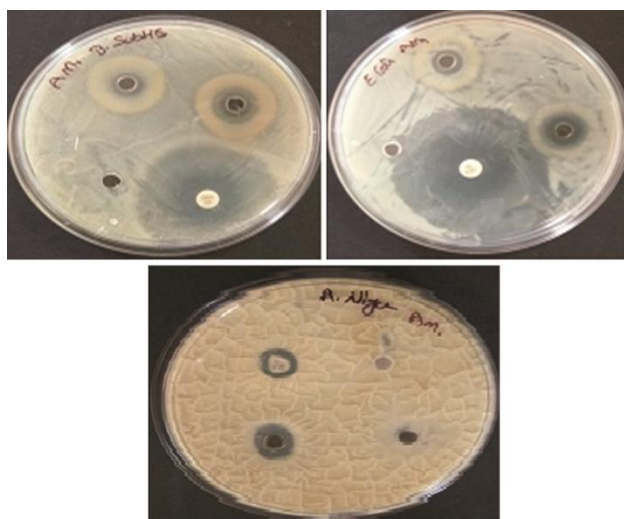


Fig. 7 — Inhibition zones for zinc oxide nanoparticles against *B. subtilis*, *E. coli*, and *A. niger*

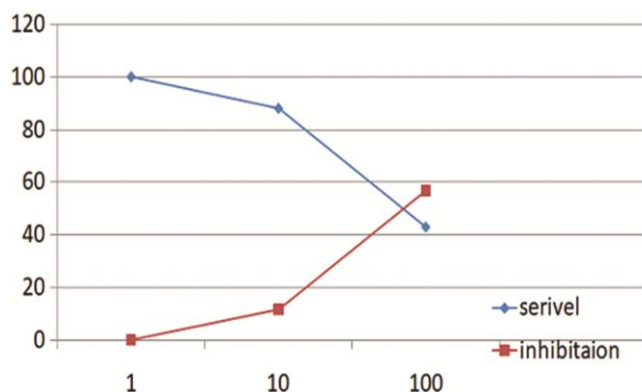


Fig. 8 — Effect of zinc oxide nanoparticles on MCF-7 Cell Viability for 24 h incubation time

Table 4 — Dose- response of zinc oxide nanoparticles on the MCF-7 cell line

Conc (µg/mL)	% Cell survival	% Cell inhibition
1	42.9334	57.0676
10	88.23529	11.76471
100	100.3512	0.35119

zones are 12 mm and 20, 21 mm the highest potency observed towards these microbes.

#### Cytotoxic studies of ZnO

The synthesized nanoparticles are screened for their cytotoxicity (MCF-7, cell lines)<sup>38-42</sup> (Fig. 8). From the data, it is observed that the particles displayed their cytotoxic activities as  $IC_{50}$  ( $\mu$ g/mL) against breast cancer MCF-7, The  $IC_{50}$  values of the nanoparticles are listed in the (table 4).

The above table liberated the high potent nature of particles. The cell survival is decreasing and % of cell inhibition is increasing with the increasing concentration of the nanoparticles compound.

Based on the above data the concentration of the sample is increasing, the inhibition rate also increases.

#### Conclusion

In the present study on “synthesis of zinc oxide nanoparticles using chemical method and their antifungal activity”, zinc oxide nanoparticles were synthesized using zinc nitrate. Synthesis conditions were optimized and resultant nanopowder was characterized using UV-Visible spectroscopy, XRD, FESEM. Morphological analysis report particle size range of 12–32 nm and also revealed that the nanoparticles are present in the form of aggregates. While studying the effect of nanoparticles for their antifungal potential, these showed activity against 2 bacterial pathogens and 1 fungal pathogen. It could be

utilized for developing antifungal agents for commercial use in the field of agriculture. This study conclusively reports a synthesis of zinc oxide nanoparticles. Such studies have the potential for developing good fungicidal formulations having nanoparticles. The cytotoxicity activities of zinc oxide nanoparticles screened by MTT assay. We have screened for one type of cancer cell line, viz., MCF-7 (breast cancer), zinc oxide obtained  $IC_{50}$  values in the range of 45-55  $\mu$ g/mL for MCF-7 cell line most of these nanoparticles are in cytotoxic activity.

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